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/106654

(54) Title: XYLANASES, NUCLEIC ADICS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

(57) Abstract: The invention relates to xylanases and to polynucleotides encoding the xylanases. In addition, methods of designing new xylanases and methods of use thereof are also provided. The xylanases have increased activity and stability at increased pH and temperature.

XYLANASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 60/389,299, filed June 14, 2002. The aforementioned application is explicitly incorporated herein by reference in its entirety and for all purposes.

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FIELD OF THE INVENTION

This invention relates generally to enzymes, polynucleotides encoding the enzymes, the use of such polynucleotides and polypeptides and more specifically to enzymes having xylanase activity, e.g., catalyzing hydrolysis of internal β -1,4-xylosidic linkages or endo- β -1,4-glucanase linkages.

BACKGROUND

Xylanases (e.g., endo-1,4-beta-xylanase, EC 3.2.1.8) hydrolyze internal β-1,4-xylosidic linkages in xylan to produce smaller molecular weight xylose and xylooligomers. Xylans are polysaccharides formed from 1,4-β-glycoside-linked D-xylopyranoses. Xylanases are of considerable commercial value, being used in the food industry, for baking and fruit and vegetable processing, breakdown of agricultural waste, in the manufacture of animal feed and in pulp and paper production. Xylanases are formed by fungi and bacteria.

Arabinoxylanase are major non-starch polysaccharides of cereals representing 2.5 – 7.1% w/w depending on variety and growth conditions. The physicochemical properties of this polysaccharide are such that it gives rise to viscous solutions or even gels under oxidative conditions. In addition, arabinoxylans have high water-binding capacity and may have a role in protein foam stability. All of these characteristics present problems for several industries including brewing, baking, animal nutrition and paper manufacturing. In brewing applications, the presence of xylan results in wort filterability and haze formation issues. In baking applications (especially for cookies and crackers), these arabinoxylans create sticky doughs that are difficult to machine and reduce biscuit size. In addition, this carbohydrate is implicated in rapid rehydration of the baked product resulting in loss of crispiness and reduced shelf-life. For monogastric animal feed applications with cereal diets, arabinoxylan is a major contributing factor to viscosity of gut contents and thereby adversely affects the digestibility of the feed and animal growth rate. For ruminant animals, these

polysaccharides represent substantial components of fiber intake and more complete digestion of arabinoxylans would facilitate higher feed conversion efficiencies.

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Xylanases are currently used as additives (dough conditioners) in dough processing for the hydrolysis of water soluble arabinoxylan. In baking applications (especially for cookies and crackers), arabinoxylan creates sticky doughs that are difficult to machine and reduce biscuit size. In addition, this carbohydrate is implicated in rapid rehydration of the baked product resulting in loss of crispiness and reduced shelf-life.

The enhancement of xylan digestion in animal feed may improve the availability and digestibility of valuable carbohydrate and protein feed nutrients. For monogastric animal feed applications with cereal diets, arabinoxylan is a major contributing factor to viscosity of gut contents and thereby adversely affects the digestibility of the feed and animal growth rate. For ruminant animals, these polysaccharides represent substantial components of fiber intake and more complete digestion would facilitate higher feed conversion efficiencies. It is desirable for animal feed xylanases to be active in the animal stomach. This requires a feed enzyme to have high activity at 37 °C and at low pH for monogastrics (pH 2-4) and near neutral pH for ruminants (pH 6.5-7). The enzyme should also possess resistance to animal gut xylanases and stability at the higher temperatures involved in feed pelleting. As such, there is a need in the art for xylanase feed additives for monogastric feed with high specific activity, activity at 35-40°C and pH 2-4, half life greater than 30 minutes in SGF and a half-life > 5 minutes at 85°C in formulated state. For ruminant feed, there is a need for xylanase feed additives that have a high specific activity, activity at 35-40°C and pH 6.5-7.0, half life greater than 30 minutes in SRF and stability as a concentrated dry powder.

Xylanases are also used in a number of other applications. For example,
xylanases are used in improving the quality and quantity of milk protein production in lactating cows (see, for example, Kung, L., et al, J. Dairy Science, 2000 Jan 83:115-122), increasing the amount of soluble saccharides in the stomach and small intestine of pigs (see, for example, van der Meulen, J. et al, Arch. Tieremahr, 2001 54:101-115), improving late egg production efficiency and egg yields in hens (see, for example, Jaroni, D., et al, Poult.
Sci., 1999 June 78:841-847). Additionally, xylanases have been shown to be useful in biobleaching and treatment of chemical pulps (see, for example, U.S. Pat. No. 5,202,249), biobleaching and treatment of wood or paper pulps (see, for example, U.S. Pat. Nos. 5,179,021, 5,116,746, 5,407,827, 5,405,769, 5,395,765, 5,369,024, 5,457,045, 5,434,071,

5,498,534, 5,591,304, 5,645,686, 5,725,732, 5,759,840, 5,834,301, 5,871,730 and 6,057,438) in reducing lignin in wood and modifying wood (see, for example, U.S. Pat. Nos. 5,486,468 and 5,770,012) as flour, dough and bread improvers (see, for example, U.S. Pat. Nos. 5,108,765 and 5,306,633) as feed additives and/or supplements, as set forth above (see, for example, U.S. Pat. Nos. 5,432,074, 5,429,828, 5,612,055, 5,720,971, 5,981,233, 5,948,667, 6,099,844, 6,132,727 and 6,132,716), in manufacturing cellulose solutions (see, for example, U.S. Pat. No.5,760,211). Detergent compositions having xylanase activity are used for fruit, vegetables and/or mud and clay compounds (see, for example, U.S. Pat. No. 5,786,316).

Xylanases are also useful in a method of use and composition of a carbohydrase and/or a xylanase for the manufacture of an agent for the treatments and/or prophylaxis of coccidiosis. The manufactured agent can be in the form of a cereal-based animal feed. (see, for example, U.S. Pat. No. 5,624,678) Additional uses for xylanases include use in the production of water soluble dietary fiber (see, for example, U.S. Pat. No. 5,622,738), in improving the filterability, separation and production of starch (see, for example, U.S. Pat. Nos. 4,960,705 and 5,023,176), in the beverage industry in improving filterability of wort or beer (see, for example, U.S. Pat. No. 4,746,517), in an enzyme composition for promoting the secretion of milk of livestock and improving the quality of the milk (see, for example, U.S. Pat. No. 4,144,354), in reducing viscosity of plant material (see, for example, U.S. Pat. No. 5,874,274), in increasing viscosity or gel strength of food products such as jam, marmalade, jelly, juice, paste, soup, salsa, etc. (see, for example, U.S. Pat. No. 6,036,981). Xylanases may also be used in hydrolysis of hemicellulose for which it is selective, particularly in the presence of cellulose. Additionally, the cellulase rich retentate is suitable for the hydrolysis of cellulose (see, for example, U.S. Pat. No. 4,725,544).

Various uses of xylanases include the production of ethanol (see, for example, PCT Application Nos. WO0043496 and WO8100857), in transformation of a microbe that produces ethanol (see, for example, PCT Application No. WO99/46362), in production of oenological tannins and enzymatic composition (see, for example, PCT Application No. WO0164830), in stimulating the natural defenses of plants (see, for example, PCT Application No. WO0130161), in production of sugars from hemicellulose substrates (see, for example, PCT Application No. WO9203541), in the cleaning of fruit, vegetables, mud or clay containing soils (see, for example, PCT Application No. WO9613568), in cleaning beer filtration membranes (see, for example, PCT Application No. WO9623579), in a method of killing or inhibiting microbial cells (see, for example, PCT Application No. WO9732480) and in determining the characteristics of process waters from wood pulp

bleaching by using the ratios of two UV absorption measurements and comparing the spectra (see, for example, PCT Application No. WO9840721).

With regard to xylanases used in the paper and pulp industry, xylanases have been isolated from many sources. In particular, see U.S. Patents No. 6,083,733 and 6,140,095 and 6,346,407. In particular, it is noted that U.S. Patents No. 6,140,095 addresses alkali-tolerant xylanases. However, it is noted that there remains a need in the art for xylanases to be used in the paper and pulp industry where the enzyme is active in the temperature range of 65°C to 75°C and at a pH of approximately 10. Additionally, an enzyme of the invention useful in the paper and pulp industry would decrease the need for bleaching chemicals, such as chlorine dioxide.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

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SUMMARY OF THE INVENTION

The invention provides isolated or recombinant nucleic acids comprising a nucleic acid sequence having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 20 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid of the invention, e.g., SEQ ID NO:1, SEO ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ 25 ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, 30 SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEO ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEO ID NO:89, SEO ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEO ID

NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID 10 NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID 15 NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEO ID NO:285, SEO ID NO:287, SEO ID NO:289, SEO ID NO:291, SEO ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID 20 NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEO ID NO:345, SEO ID NO:347, SEO ID NO:349, SEQ ID NO:351, SEO ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID 25 NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379, over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 30 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2050, 2100, 2200, 2250, 2300, 2350, 2400, 2450, 2500, or more residues, encodes at least one polypeptide having a xylanase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

Exemplary nucleic acids of the invention also include isolated or recombinant nucleic acids encoding a polypeptide having a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID 5 NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, 10 SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132; 15 SEQ ID NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; SEQ ID NO:142; SEQ ID NO:144; NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID 20 NO:184, SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, SEQ ID 25 NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, SEQ ID NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278, SEQ ID NO:280, SEQ ID NO:282, SEQ ID 30 NO:284, SEQ ID NO:286, SEQ ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, SEQ ID NO:320, SEQ ID NO:322, SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID

NO:334, SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376, SEQ ID NO:378 or SEQ ID NO:380, and subsequences thereof and variants thereof. In one aspect, the polypeptide has a xylanase activity.

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In one aspect, the invention also provides xylanase-encoding nucleic acids with a common novelty in that they are derived from mixed cultures. The invention provides xylanase-encoding nucleic acids isolated from mixed cultures comprising a nucleic acid sequence having at least about 10, 15, 20, 25, 30, 35, 40, 45, 50%, 51%, 52%, 53%, 54%, 10 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid of the invention, e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ 15 ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, 20 SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID 25 NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEO ID NO:133, SEO ID NO:135, SEO ID NO:137, SEO ID NO:139, SEO ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID 30 NO:161, SEO ID NO:163, SEO ID NO:165, SEO ID NO:167, SEO ID NO:169, SEO ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID

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In one aspect, the invention also provides xylanase-encoding nucleic acids with a common novelty in that they are derived from an environmental source, e.g., mixed environmental sources, a bacterial source and/or an archaeal source, see Table 3, below. In one aspect, the invention provides xylanase-encoding nucleic acids isolated from an environmental source, e.g., a mixed environmental source, a bacterial source and/or an archaeal source, comprising a nucleic acid sequence having at least about 10, 15, 20, 25, 30, 35, 40, 45, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid of the invention over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200 or more, residues, wherein the nucleic acid encodes at least one polypeptide having a xylanase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

In one aspect, the invention also provides xylanase-encoding nucleic acids with a common novelty in that they are derived from a common glycosidase family, e.g., family 5, 6, 8, 10, 11, 26 or 30, as set forth in Table 5, below.

In one aspect, the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default.

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Another aspect of the invention is an isolated or recombinant nucleic acid including at least 10 consecutive bases of a nucleic acid sequence of the invention, sequences substantially identical thereto, and the sequences complementary thereto.

In one aspect, the xylanase activity comprises catalyzing hydrolysis of internal β -1,4-xylosidic linkages. In one aspect, the xylanase activity comprises an endo-1,4-beta-xylanase activity.

In one aspect, the xylanase activity comprises hydrolyzing a xylan to produce a smaller molecular weight xylose and xylo-oligomer. In one aspect, the xylan comprises an arabinoxylan, such as a water soluble arabinoxylan. The water soluble arabinoxylan can comprise a dough or a bread product.

In one aspect, the xylanase activity comprises hydrolyzing polysaccharides comprising 1,4- β -glycoside-linked D-xylopyranoses. In one aspect, the xylanase activity comprises hydrolyzing hemicelluloses. In one aspect, the xylanase activity comprises hydrolyzing hemicelluloses in a wood or paper pulp or a paper product. In one aspect, the invention provides methods for reducing lignin in a wood or wood product comprising contacting the wood or wood product with a polypeptide of the invention.

In one aspect, the xylanase activity comprises catalyzing hydrolysis of xylans in a beverage or a feed or a food product. The feed or food product can comprise a cereal-based animal feed, a wort or a beer, a milk or a milk product, a fruit or a vegetable. In one aspect, the invention provides a food, feed or beverage or a beverage precursor comprising a polypeptide of the invention. The food can be a dough or a bread product. The beverage or a beverage precursor can be a beer or a wort.

In one aspect, the invention provides methods of dough conditioning comprising contacting a dough or a bread product with at least one polypeptide of the invention under conditions sufficient for conditioning the dough. In one aspect, the invention provides methods of beverage production comprising administration of at least one polypeptide of the invention to a beverage or a beverage precursor under conditions sufficient for decreasing the viscosity of the beverage.

In one aspect, the xylanase activity comprises catalyzing hydrolysis of xylans in a cell, e.g., a plant cell or a microbial cell.

In one aspect, the isolated or recombinant nucleic acid encodes a polypeptide having a xylanase activity that is thermostable. The polypeptide can retain a xylanase activity under conditions comprising a temperature range of between about 37°C to about 95°C; between about 55°C to about 85°C, between about 70°C to about 95°C, or, between about 90°C to about 95°C.

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In another aspect, the isolated or recombinant nucleic acid encodes a polypeptide having a xylanase activity that is thermotolerant. The polypeptide can retain a xylanase activity after exposure to a temperature in the range from greater than 37°C to about 95°C or anywhere in the range from greater than 55°C to about 85°C. The polypeptide can retain a xylanase activity after exposure to a temperature in the range between about 1°C to about 5°C, between about 5°C to about 15°C, between about 15°C to about 25°C, between about 25°C to about 37°C, between about 37°C to about 95°C, between about 55°C to about 85°C, between about 70°C to about 75°C, or between about 90°C to about 95°C, or more. In one aspect, the polypeptide retains a xylanase activity after exposure to a temperature in the range from greater than 90°C to about 95°C at pH 4.5.

The invention provides isolated or recombinant nucleic acids comprising a sequence that hybridizes under stringent conditions to a nucleic acid comprising a sequence of the invention, e.g., a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID

NO:155, SEO ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEO ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID 5 NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEO ID NO:207, SEO ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEO ID NO:227, SEO ID NO:229, SEO ID NO:231, SEO ID NO:233, SEO ID NO:235, SEO ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID 10 NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEO ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID 15 NO:295, SEO ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEO ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID 20 NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379, or fragments or subsequences thereof. In one aspect, the nucleic acid encodes a polypeptide having a xylanase activity. The nucleic acid 25 can be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200 or more residues in length or the full length of the gene or transcript. In one aspect, the stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

The invention provides a nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a xylanase activity, wherein the probe comprises at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more, consecutive bases of a sequence comprising a sequence of the invention, or fragments or subsequences

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thereof, wherein the probe identifies the nucleic acid by binding or hybridization. The probe can comprise an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 consecutive bases of a sequence comprising a sequence of the invention, or fragments or subsequences thereof.

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The invention provides a nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a xylanase activity, wherein the probe comprises a nucleic acid comprising a sequence at least about 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more residues having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to a nucleic acid of the invention, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

The probe can comprise an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 consecutive bases of a nucleic acid sequence of the invention, or a subsequence thereof.

The invention provides an amplification primer pair for amplifying a nucleic acid encoding a polypeptide having a xylanase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence of the invention, or fragments or subsequences thereof. One or each member of the amplification primer sequence pair can comprise an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence, or about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more consecutive bases of the sequence.

The invention provides amplification primer pairs, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of a nucleic acid of the invention, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of the complementary strand of the first member.

The invention provides xylanase-encoding nucleic acids generated by amplification, e.g., polymerase chain reaction (PCR), using an amplification primer pair of the invention. The invention provides xylanases generated by amplification, e.g., polymerase chain reaction (PCR), using an amplification primer pair of the invention. The invention

provides methods of making a xylanase by amplification, e.g., polymerase chain reaction (PCR), using an amplification primer pair of the invention. In one aspect, the amplification primer pair amplifies a nucleic acid from a library, e.g., a gene library, such as an environmental library.

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The invention provides methods of amplifying a nucleic acid encoding a polypeptide having a xylanase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence of the invention, or fragments or subsequences thereof.

The invention provides expression cassettes comprising a nucleic acid of the invention or a subsequence thereof. In one aspect, the expression cassette can comprise the nucleic acid that is operably linked to a promoter. The promoter can be a viral, bacterial, mammalian or plant promoter. In one aspect, the plant promoter can be a potato, rice, corn, wheat, tobacco or barley promoter. The promoter can be a constitutive promoter. The constitutive promoter can comprise CaMV35S. In another aspect, the promoter can be an inducible promoter. In one aspect, the promoter can be a tissue-specific promoter or an environmentally regulated or a developmentally regulated promoter. Thus, the promoter can be, e.g., a seed-specific, a leaf-specific, a root-specific, a stem-specific or an abscission-induced promoter. In one aspect, the expression cassette can further comprise a plant or plant virus expression vector.

The invention provides cloning vehicles comprising an expression cassette (e.g., a vector) of the invention or a nucleic acid of the invention. The cloning vehicle can be a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome. The viral vector can comprise an adenovirus vector, a retroviral vector or an adeno-associated viral vector. The cloning vehicle can comprise a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).

The invention provides transformed cell comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention, or a cloning vehicle of the invention. In one aspect, the transformed cell can be a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell. In one aspect, the plant cell can be a cereal, a potato, wheat, rice, corn, tobacco or barley cell.

The invention provides transgenic non-human animals comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. In one aspect, the animal is a mouse.

The invention provides transgenic plants comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. The transgenic plant can be a cereal plant, a corn plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant or a tobacco plant.

The invention provides transgenic seeds comprising a nucleic acid of the invention or an expression cassette (e.g., a vector) of the invention. The transgenic seed can be a cereal plant, a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a peanut or a tobacco plant seed.

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The invention provides an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention. The invention provides methods of inhibiting the translation of a xylanase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention. In one aspect, the antisense oligonucleotide is between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 bases in length.

The invention provides methods of inhibiting the translation of a xylanase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a nucleic acid of the invention. The invention provides double-stranded inhibitory RNA (RNAi) molecules comprising a subsequence of a sequence of the invention. In one aspect, the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length. The invention provides methods of inhibiting the expression of a xylanase in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory RNA (iRNA), wherein the RNA comprises a subsequence of a sequence of the invention.

The invention provides an isolated or recombinant polypeptide comprising an amino acid sequence having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary polypeptide or peptide of the invention over a region of at least about 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350 or more residues, or over the full length of the polypeptide, and the sequence identities are determined

by analysis with a sequence comparison algorithm or by a visual inspection. Exemplary polypeptide or peptide sequences of the invention include SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID 5 NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, 10 SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132; SEQ ID 15 NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; SEQ ID NO:142; SEQ ID NO:144; NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, 20 SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:242, SEQ ID NO:244, 25 SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, SEQ ID NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278, SEQ ID NO:280, SEQ ID NO:282, SEQ ID NO:284, 30 SEQ ID NO:286, SEQ ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, SEQ ID NO:320, SEQ ID NO:322, SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID NO:334,

SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376, SEQ ID NO:378 or SEQ ID NO:380, and subsequences thereof and variants thereof. Exemplary polypeptides also include fragments of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600 or more residues in length, or over the full length of an enzyme. Exemplary polypeptide or peptide sequences of the invention include sequence encoded by a nucleic acid of the invention.

10 Exemplary polypeptide or peptide sequences of the invention include polypeptides or peptides specifically bound by an antibody of the invention. In one aspect, a polypeptide of the invention has at least one xylanase activity.

Another aspect of the invention provides an isolated or recombinant polypeptide or peptide including at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 or more consecutive bases of a polypeptide or peptide sequence of the invention, sequences substantially identical thereto, and the sequences complementary thereto. The peptide can be, e.g., an immunogenic fragment, a motif (e.g., a binding site), a signal sequence, a prepro sequence or an active site.

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The invention provides isolated or recombinant nucleic acids comprising a sequence encoding a polypeptide having a xylanase activity and a signal sequence, wherein the nucleic acid comprises a sequence of the invention. The signal sequence can be derived from another xylanase or a non-xylanase (a heterologous) enzyme. The invention provides isolated or recombinant nucleic acids comprising a sequence encoding a polypeptide having a xylanase activity, wherein the sequence does not contain a signal sequence and the nucleic acid comprises a sequence of the invention.

In one aspect, the xylanase activity comprises catalyzing hydrolysis of internal β -1,4-xylosidic linkages. In one aspect, the xylanase activity comprises an endo-1,4-beta-xylanase activity. In one aspect, the xylanase activity comprises hydrolyzing a xylan to produce a smaller molecular weight xylose and xylo-oligomer. In one aspect, the xylan comprises an arabinoxylan, such as a water soluble arabinoxylan. The water soluble arabinoxylan can comprise a dough or a bread product.

In one aspect, the xylanase activity comprises hydrolyzing polysaccharides comprising 1,4-β-glycoside-linked D-xylopyranoses. In one aspect, the xylanase activity

comprises hydrolyzing hemicelluloses. In one aspect, the xylanase activity comprises hydrolyzing hemicelluloses in a wood or paper pulp or a paper product.

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In one aspect, the xylanase activity comprises catalyzing hydrolysis of xylans in a feed or a food product. The feed or food product can comprise a cereal-based animal feed, a wort or a beer, a milk or a milk product, a fruit or a vegetable.

In one aspect, the xylanase activity comprises catalyzing hydrolysis of xylans in a cell, e.g., a plant cell or a microbial cell.

In one aspect, the xylanase activity is thermostable. The polypeptide can retain a xylanase activity under conditions comprising a temperature range of between about 1°C to about 5°C, between about 5°C to about 5°C, between about 5°C, between about 37°C to about 95°C, between about 55°C to about 85°C, between about 70°C to about 75°C, or between about 90°C to about 95°C, or more. In another aspect, the xylanase activity can be thermotolerant. The polypeptide can retain a xylanase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, or in the range from greater than 55°C to about 85°C. In one aspect, the polypeptide can retain a xylanase activity after exposure to a temperature in the range from greater than 90°C to about 95°C at pH 4.5.

In one aspect, the isolated or recombinant polypeptide can comprise the polypeptide of the invention that lacks a signal sequence. In one aspect, the isolated or recombinant polypeptide can comprise the polypeptide of the invention comprising a heterologous signal sequence, such as a heterologous xylanase or non-xylanase signal sequence.

In one aspect, the invention provides chimeric proteins comprising a first domain comprising a signal sequence of the invention and at least a second domain. The protein can be a fusion protein. The second domain can comprise an enzyme. The enzyme can be a xylanase.

The invention provides chimeric polypeptides comprising at least a first domain comprising signal peptide (SP), a prepro sequence and/or a catalytic domain (CD) of the invention and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP), prepro sequence and/or catalytic domain (CD). In one aspect, the heterologous polypeptide or peptide is not a xylanase. The heterologous polypeptide or peptide can be amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP), prepro sequence and/or catalytic domain (CD).

The invention provides isolated or recombinant nucleic acids encoding a chimeric polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide (SP), a prepro domain and/or a catalytic domain (CD) of the invention and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP), prepro domain and/or catalytic domain (CD).

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The invention provides isolated or recombinant signal sequences (e.g., signal peptides) consisting of a sequence as set forth in residues 1 to 14, 1 to 15, 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 10 28, 1 to 30, 1 to 31, 1 to 32, 1 to 33, 1 to 34, 1 to 35, 1 to 36, 1 to 37, 1 to 38, 1 to 40, 1 to 41, 1 to 42, 1 to 43 or 1 to 44, of a polypeptide of the invention, e.g., SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70. SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132; SEO ID NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; SEO ID NO:142; SEO ID NO:144; NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEO ID NO:192, SEO ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:242, SEQ ID NO:244,

SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, SEQ ID NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278, SEQ ID NO:280, SEQ ID NO:282, SEQ ID NO:284, SEQ ID NO:286, SEQ ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, SEQ ID NO:320, SEQ ID NO:322, SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID NO:334, SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376, SEQ ID NO:378 or SEQ ID NO:380.

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In one aspect, the xylanase activity comprises a specific activity at about 37°C in the range from about 1 to about 1200 units per milligram of protein, or, about 100 to about 1000 units per milligram of protein. In another aspect, the xylanase activity comprises a specific activity from about 100 to about 1000 units per milligram of protein, or, from about 500 to about 750 units per milligram of protein. Alternatively, the xylanase activity comprises a specific activity at 37°C in the range from about 1 to about 750 units per milligram of protein, or, from about 500 to about 1200 units per milligram of protein. In one aspect, the xylanase activity comprises a specific activity at 37°C in the range from about 1 to about 500 units per milligram of protein, or, from about 750 to about 1000 units per milligram of protein. In another aspect, the xylanase activity comprises a specific activity at 37°C in the range from about 1 to about 250 units per milligram of protein. Alternatively, the xylanase activity comprises a specific activity at 37°C in the range from about 1 to about 100 units per milligram of protein. In another aspect, the thermotolerance comprises retention of at least half of the specific activity of the xylanase at 37°C after being heated to the elevated temperature. Alternatively, the thermotolerance can comprise retention of specific activity at 37°C in the range from about 1 to about 1200 units per milligram of protein, or, from about 500 to about 1000 units per milligram of protein, after being heated to the elevated temperature. In another aspect, the thermotolerance can comprise retention of specific activity at 37°C in the range from about 1 to about 500 units per milligram of protein after being heated to the elevated temperature.

The invention provides the isolated or recombinant polypeptide of the invention, wherein the polypeptide comprises at least one glycosylation site. In one aspect, glycosylation can be an N-linked glycosylation. In one aspect, the polypeptide can be glycosylated after being expressed in a *P. pastoris* or a *S. pombe*.

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In one aspect, the polypeptide can retain a xylanase activity under conditions comprising about pH 6.5, pH 6, pH 5.5, pH 5, pH 4.5 or pH 4. In another aspect, the polypeptide can retain a xylanase activity under conditions comprising about pH 7, pH 7.5 pH 8.0, pH 8.5, pH 9, pH 9.5, pH 10, pH 10.5 or pH 11. In one aspect, the polypeptide can retain a xylanase activity after exposure to conditions comprising about pH 6.5, pH 6, pH 5.5, pH 5, pH 4.5 or pH 4. In another aspect, the polypeptide can retain a xylanase activity after exposure to conditions comprising about pH 7, pH 7.5 pH 8.0, pH 8.5, pH 9, pH 9.5, pH 10, pH 10.5 or pH 11.

The invention provides protein preparations comprising a polypeptide of the invention, wherein the protein preparation comprises a liquid, a solid or a gel.

The invention provides heterodimers comprising a polypeptide of the invention and a second protein or domain. The second member of the heterodimer can be a different phospholipase, a different enzyme or another protein. In one aspect, the second domain can be a polypeptide and the heterodimer can be a fusion protein. In one aspect, the second domain can be an epitope or a tag. In one aspect, the invention provides homodimers comprising a polypeptide of the invention.

The invention provides immobilized polypeptides having a xylanase activity, wherein the polypeptide comprises a polypeptide of the invention, a polypeptide encoded by a nucleic acid of the invention, or a polypeptide comprising a polypeptide of the invention and a second domain. In one aspect, the polypeptide can be immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.

The invention provides arrays comprising an immobilized nucleic acid of the invention. The invention provides arrays comprising an antibody of the invention.

The invention provides isolated or recombinant antibodies that specifically bind to a polypeptide of the invention or to a polypeptide encoded by a nucleic acid of the invention. The antibody can be a monoclonal or a polyclonal antibody. The invention provides hybridomas comprising an antibody of the invention, e.g., an antibody that specifically binds to a polypeptide of the invention or to a polypeptide encoded by a nucleic acid of the invention.

The invention provides method of isolating or identifying a polypeptide having a xylanase activity comprising the steps of: (a) providing an antibody of the invention; (b) providing a sample comprising polypeptides; and (c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a xylanase activity.

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The invention provides methods of making an anti-xylanase antibody comprising administering to a non-human animal a nucleic acid of the invention or a polypeptide of the invention or subsequences thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-xylanase antibody. The invention provides methods of making an anti-xylanase immune comprising administering to a non-human animal a nucleic acid of the invention or a polypeptide of the invention or subsequences thereof in an amount sufficient to generate an immune response.

The invention provides methods of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid of the invention operably linked to a promoter; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide. In one aspect, the method can further comprise transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.

The invention provides methods for identifying a polypeptide having a xylanase activity comprising the following steps: (a) providing a polypeptide of the invention; or a polypeptide encoded by a nucleic acid of the invention; (b) providing a xylanase substrate; and (c) contacting the polypeptide or a fragment or variant thereof of step (a) with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a xylanase activity.

The invention provides methods for identifying a xylanase substrate comprising the following steps: (a) providing a polypeptide of the invention; or a polypeptide encoded by a nucleic acid of the invention; (b) providing a test substrate; and (c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a xylanase substrate.

The invention provides methods of determining whether a test compound specifically binds to a polypeptide comprising the following steps: (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid comprises a nucleic acid of the invention, or, providing a polypeptide of the invention; (b) providing a test compound; (c) contacting the polypeptide with the test compound; and (d) determining whether the test compound of step (b) specifically binds to the polypeptide.

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The invention provides methods for identifying a modulator of a xylanase activity comprising the following steps: (a) providing a polypeptide of the invention or a polypeptide encoded by a nucleic acid of the invention; (b) providing a test compound; (c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the xylanase, wherein a change in the xylanase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the xylanase activity. In one aspect, the xylanase activity can be measured by providing a xylanase substrate and detecting a decrease: in the amount of the substrate or an increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product. A decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of xylanase activity. An increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of xylanase activity.

The invention provides computer systems comprising a processor and a data storage device wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid sequence of the invention (e.g., a polypeptide encoded by a nucleic acid of the invention). In one aspect, the computer system can further comprise a sequence comparison algorithm and a data storage device having at least one reference sequence stored thereon. In another aspect, the sequence comparison algorithm comprises a computer program that indicates polymorphisms. In one aspect, the computer system can further comprise an identifier that identifies one or more features in said sequence. The invention provides computer readable media having stored thereon a polypeptide sequence or a nucleic acid sequence of the invention. The invention provides methods for identifying a feature in a sequence comprising the steps of: (a) reading the sequence using a computer program which

identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence of the invention; and (b) identifying one or more features in the sequence with the computer program. The invention provides methods for comparing a first sequence to a second sequence comprising the steps of: (a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence of the invention; and (b) determining differences between the first sequence and the second sequence with the computer program. The step of determining differences between the first sequence and the second sequence can further comprise the step of identifying polymorphisms. In one aspect, the method can further comprise an identifier that identifies one or more features in a sequence. In another aspect, the method can comprise reading the first sequence using a computer program and identifying one or more features in the sequence.

The invention provides methods for isolating or recovering a nucleic acid encoding a polypeptide having a xylanase activity from an environmental sample comprising the steps of: (a) providing an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a xylanase activity, wherein the primer pair is capable of amplifying a nucleic acid of the invention; (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and, (c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or recovering a nucleic acid encoding a polypeptide having a xylanase activity from an environmental sample. One or each member of the amplification primer sequence pair can comprise an oligonucleotide comprising at least about 10 to 50 consecutive bases of a sequence of the invention. In one aspect, the amplification primer sequence pair is an amplification pair of the invention.

The invention provides methods for isolating or recovering a nucleic acid encoding a polypeptide having a xylanase activity from an environmental sample comprising the steps of: (a) providing a polynucleotide probe comprising a nucleic acid of the invention or a subsequence thereof; (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a); (c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and (d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a),

thereby isolating or recovering a nucleic acid encoding a polypeptide having a xylanase activity from an environmental sample. The environmental sample can comprise a water sample, a liquid sample, a soil sample, an air sample or a biological sample. In one aspect, the biological sample can be derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.

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The invention provides methods of generating a variant of a nucleic acid encoding a polypeptide having a xylanase activity comprising the steps of: (a) providing a template nucleic acid comprising a nucleic acid of the invention; and (b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid. In one aspect, the method can further comprise expressing the variant nucleic acid to generate a variant xylanase polypeptide. The modifications, additions or deletions can be introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly (e.g., GeneReassembly™, see, e.g., U.S. Patent No. 6,537,776), gene site saturated mutagenesis (GSSMTM), synthetic ligation reassembly (SLR) or a combination thereof. In another aspect, the modifications, additions or deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restrictionpurification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

In one aspect, the method can be iteratively repeated until a xylanase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced. In one aspect, the variant xylanase polypeptide is thermotolerant, and retains some activity after being exposed to an elevated temperature. In another aspect, the variant xylanase polypeptide has increased glycosylation as compared to the xylanase encoded by a template nucleic acid. Alternatively, the variant xylanase polypeptide has a xylanase activity under a high temperature, wherein the xylanase encoded by the template nucleic acid is not active under the high temperature. In one aspect, the method can be iteratively repeated until a xylanase coding sequence having an altered codon usage from that of the template nucleic acid is produced. In another aspect, the

method can be iteratively repeated until a xylanase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.

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In one aspect, the invention provides isolated or recombinant nucleic acids comprising a sequence as set forth in SEQ ID NO: 189, wherein SEQ ID NO: 189 contains one or more of the following mutations: the nucleotides at positions 22 to 24 are TTC, the nucleotides at positions 31 to 33 are CAC, the nucleotides at positions 34 to 36 are TTG, the nucleotides at positions 49 to 51 are ATA, the nucleotides at positions 31 to 33 are CAT, the nucleotides at positions 67 to 69 are ACG, the nucleotides at positions 178 to 180 are CAC, the nucleotides at positions 190 to 192 are TGT, the nucleotides at positions 190 to 192 are GTA, the nucleotides at positions 190 to 192 are GTG, the nucleotides at positions 202 to 204 are GCT, the nucleotides at positions 235 to 237 are CCA, or the nucleotides at positions 235 to 237 are CCC. In one aspect, the invention provides methods for making a nucleic acid comprising this sequence, wherein the mutations in SEQ ID NO: 189 are obtained by gene site saturated mutagenesis (GSSMTM).

In one aspect, the invention provides isolated or recombinant nucleic acids comprising SEQ ID NO: 190, wherein SEQ ID NO: 190 contains one or more of the following mutations: the aspartic acid at amino acid position 8 is phenylalanine, the glutamine at amino acid position 11 is histidine, the asparagine at amino acid position 12 is leucine, the glycine at amino acid position 17 is isoleucine, the threonine at amino acid position 23 is threonine encoded by a codon other than the wild type codon, the glycine at amino acid position 60 is histidine, the proline at amino acid position 64 is cysteine, the proline at amino acid position 64 is valine, the serine at amino acid position 65 is valine, the glycine at amino acid position 68 is isoleucine, the glycine at amino acid position 68 is alanine, or the valine at amino acid position 79 is proline.

The invention provides methods for modifying codons in a nucleic acid encoding a polypeptide having a xylanase activity to increase its expression in a host cell, the method comprising the following steps: (a) providing a nucleic acid of the invention encoding a polypeptide having a xylanase activity; and, (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

The invention provides methods for modifying codons in a nucleic acid encoding a polypeptide having a xylanase activity; the method comprising the following steps: (a) providing a nucleic acid of the invention; and, (b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a xylanase.

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The invention provides methods for modifying codons in a nucleic acid encoding a polypeptide having a xylanase activity to increase its expression in a host cell, the method comprising the following steps: (a) providing a nucleic acid of the invention encoding a xylanase polypeptide; and, (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.

The invention provides methods for modifying a codon in a nucleic acid encoding a polypeptide having a xylanase activity to decrease its expression in a host cell, the method comprising the following steps: (a) providing a nucleic acid of the invention; and (b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell. In one aspect, the host cell can be a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.

The invention provides methods for producing a library of nucleic acids encoding a plurality of modified xylanase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the following steps: (a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes under stringent conditions to a nucleic acid of the invention, and the nucleic acid encodes a xylanase active site or a xylanase substrate binding site; (b) providing a set of mutagenic oligonucleotides that encode naturally-occurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and, (c) using the set of mutagenic

oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified xylanase active sites or substrate binding sites. In one aspect, the method comprises mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system, gene site-saturation mutagenesis (GSSMTM), synthetic ligation reassembly (SLR), error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly (GeneReassemblyTM, U.S. Patent No. 6,537,776), gene site saturated mutagenesis (GSSMTM), synthetic ligation reassembly (SLR) and a combination thereof. In another aspect, the method comprises mutagenizing the first nucleic acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restrictionpurification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.

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The invention provides methods for making a small molecule comprising the following steps: (a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a xylanase enzyme encoded by a nucleic acid of the invention; (b) providing a substrate for at least one of the enzymes of step (a); and (c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions. The invention provides methods for modifying a small molecule comprising the following steps: (a) providing a xylanase enzyme, wherein the enzyme comprises a polypeptide of the invention, or, a polypeptide encoded by a nucleic acid of the invention, or a subsequence thereof; (b) providing a small molecule; and (c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the xylanase enzyme, thereby modifying a small molecule by a xylanase enzymatic reaction. In one aspect, the method can comprise a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the xylanase enzyme. In one aspect, the method can comprise a plurality of additional enzymes under

conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions. In another aspect, the method can further comprise the step of testing the library to determine if a particular modified small molecule that exhibits a desired activity is present within the library. The step of testing the library can further comprise the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

The invention provides methods for determining a functional fragment of a xylanase enzyme comprising the steps of: (a) providing a xylanase enzyme, wherein the enzyme comprises a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention, or a subsequence thereof; and (b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a xylanase activity, thereby determining a functional fragment of a xylanase enzyme. In one aspect, the xylanase activity is measured by providing a xylanase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product.

The invention provides methods for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps: (a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid of the invention; (b) culturing the modified cell to generate a plurality of modified cells; (c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and, (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis. In one aspect, the genetic composition of the cell can be modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene. In one aspect, the method can further comprise selecting a cell comprising a newly engineered phenotype. In another aspect, the method can comprise culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.

The invention provides methods of increasing thermotolerance or thermostability of a xylanase polypeptide, the method comprising glycosylating a xylanase polypeptide, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide of the invention; or a polypeptide encoded by a nucleic acid sequence of the invention, thereby increasing the thermotolerance or thermostability of the xylanase polypeptide. In one aspect, the xylanase specific activity can be thermostable or thermotolerant at a temperature in the range from greater than about 37°C to about 95°C.

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The invention provides methods for overexpressing a recombinant xylanase polypeptide in a cell comprising expressing a vector comprising a nucleic acid comprising a nucleic acid of the invention or a nucleic acid sequence of the invention, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

The invention provides methods of making a transgenic plant comprising the following steps: (a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a nucleic acid sequence of the invention, thereby producing a transformed plant cell; and (b) producing a transgenic plant from the transformed cell. In one aspect, the step (a) can further comprise introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts. In another aspect, the step (a) can further comprise introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment. Alternatively, the step (a) can further comprise introducing the heterologous nucleic acid sequence into the plant cell DNA using an Agrobacterium tumefaciens host. In one aspect, the plant cell can be a potato, corn, rice, wheat, tobacco, or barley cell.

The invention provides methods of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps: (a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a nucleic acid of the invention; (b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell. The invention provides methods of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps: (a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence of the invention; (b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

The invention provides methods for hydrolyzing, breaking up or disrupting a xylan-comprising composition comprising the following steps: (a) providing a polypeptide of the invention having a xylanase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a composition comprising a xylan; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the xylanase hydrolyzes, breaks up or disrupts the xylan-comprising composition. In one aspect, the composition comprises a plant cell, a bacterial cell, a yeast cell, an insect cell, or an animal cell. Thus, the composition can comprise any plant or plant part, any xylan-containing food or feed, a waste product and the like. The invention provides methods for liquefying or removing a xylan-comprising composition comprising the following steps: (a) providing a polypeptide of the invention having a xylanase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a composition comprising a xylan; and (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the xylanase removes, softens or liquefies the xylan-comprising composition.

The invention provides detergent compositions comprising a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention, wherein the polypeptide has a xylanase activity. The xylanase can be a nonsurface-active xylanase or a surface-active xylanase. The xylanase can be formulated in a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel form, a paste or a slurry form. The invention provides methods for washing an object comprising the following steps: (a) providing a composition comprising a polypeptide of the invention having a xylanase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing an object; and (c) contacting the polypeptide of step (a) and the object of step (b) under conditions wherein the composition can wash the object.

The invention provides textiles or fabrics, including, e.g., threads, comprising a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention. In one aspect, the textiles or fabrics comprise xylan-containing fibers. The invention provides methods for treating a textile or fabric (e.g., removing a stain from a composition) comprising the following steps: (a) providing a composition comprising a polypeptide of the invention having a xylanase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a textile or fabric comprising a xylan; and (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the xylanase can treat the textile or fabric (e.g., remove the stain). The invention provides methods for improving the finish of a fabric comprising the following steps: (a) providing a composition

comprising a polypeptide of the invention having a xylanase activity, or a polypeptide encoded by a nucleic acid of the invention; (b) providing a fabric; and (c) contacting the polypeptide of step (a) and the fabric of step (b) under conditions wherein the polypeptide can treat the fabric thereby improving the finish of the fabric. In one aspect, the fabric is a wool or a silk.

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The invention provides feeds or foods comprising a polypeptide of the invention, or a polypeptide encoded by a nucleic acid of the invention. The invention provides methods for hydrolyzing xylans in a feed or a food prior to consumption by an animal comprising the following steps: (a) obtaining a feed material comprising a xylanase of the invention, or a xylanase encoded by a nucleic acid of the invention; and (b) adding the polypeptide of step (a) to the feed or food material in an amount sufficient for a sufficient time period to cause hydrolysis of the xylan and formation of a treated food or feed, thereby hydrolyzing the xylans in the food or the feed prior to consumption by the animal. In one aspect, the invention provides methods for hydrolyzing xylans in a feed or a food after consumption by an animal comprising the following steps: (a) obtaining a feed material comprising a xylanase of the invention, or a xylanase encoded by a nucleic acid of the invention; (b) adding the polypeptide of step (a) to the feed or food material; and (c) administering the feed or food material to the animal, wherein after consumption, the xylanase causes hydrolysis of xylans in the feed or food in the digestive tract of the animal. The food or the feed can be, e.g., a cereal, a grain, a corn and the like.

The invention provides food or nutritional supplements for an animal comprising a polypeptide of the invention, e.g., a polypeptide encoded by the nucleic acid of the invention. In one aspect, the polypeptide in the food or nutritional supplement can be glycosylated. The invention provides edible enzyme delivery matrices comprising a polypeptide of the invention, e.g., a polypeptide encoded by the nucleic acid of the invention. In one aspect, the delivery matrix comprises a pellet. In one aspect, the polypeptide can be glycosylated. In one aspect, the xylanase activity is thermotolerant. In another aspect, the xylanase activity is thermostable.

The invention provides a food, a feed or a nutritional supplement comprising a polypeptide of the invention. The invention provides methods for utilizing a xylanase as a nutritional supplement in an animal diet, the method comprising: preparing a nutritional supplement containing a xylanase enzyme comprising at least thirty contiguous amino acids of a polypeptide of the invention; and administering the nutritional supplement to an animal to increase utilization of a xylan contained in a feed or a food ingested by the animal. The

animal can be a human, a ruminant or a monogastric animal. The xylanase enzyme can be prepared by expression of a polynucleotide encoding the xylanase in an organism selected from the group consisting of a bacterium, a yeast, a plant, an insect, a fungus and an animal. The organism can be selected from the group consisting of an S. pombe, S. cerevisiae, Pichia pastoris, Pseudomonas sp., E. coli, Streptomyces sp., Bacillus sp. and Lactobacillus sp.

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The invention provides edible enzyme delivery matrix comprising a thermostable recombinant xylanase enzyme, e.g., a polypeptide of the invention. The invention provides methods for delivering a xylanase supplement to an animal, the method comprising: preparing an edible enzyme delivery matrix in the form of pellets comprising a granulate edible carrier and a thermostable recombinant xylanase enzyme, wherein the pellets readily disperse the xylanase enzyme contained therein into aqueous media, and administering the edible enzyme delivery matrix to the animal. The recombinant xylanase enzyme can comprise a polypeptide of the invention. The granulate edible carrier can comprise a carrier selected from the group consisting of a grain germ, a grain germ that is spent of oil, a hay, an alfalfa, a timothy, a soy hull, a sunflower seed meal and a wheat midd. The edible carrier can comprise grain germ that is spent of oil. The xylanase enzyme can be glycosylated to provide thermostability at pelletizing conditions. The delivery matrix can be formed by pelletizing a mixture comprising a grain germ and a xylanase. The pelletizing conditions can include application of steam. The pelletizing conditions can comprise application of a temperature in excess of about 80°C for about 5 minutes and the enzyme retains a specific activity of at least 350 to about 900 units per milligram of enzyme.

The invention provides methods for improving texture and flavor of a dairy product comprising the following steps: (a) providing a polypeptide of the invention having a xylanase activity, or a xylanase encoded by a nucleic acid of the invention; (b) providing a dairy product; and (c) contacting the polypeptide of step (a) and the dairy product of step (b) under conditions wherein the xylanase can improve the texture or flavor of the dairy product. In one aspect, the dairy product comprises a cheese or a yogurt. The invention provides dairy products comprising a xylanase of the invention, or is encoded by a nucleic acid of the invention.

The invention provides methods for improving the extraction of oil from an oil-rich plant material comprising the following steps: (a) providing a polypeptide of the invention having a xylanase activity, or a xylanase encoded by a nucleic acid of the invention; (b) providing an oil-rich plant material; and (c) contacting the polypeptide of step (a) and the oil-rich plant material. In one aspect, the oil-rich plant material comprises an oil-

rich seed. The oil can be a soybean oil, an olive oil, a rapeseed (canola) oil or a sunflower oil.

The invention provides methods for preparing a fruit or vegetable juice, syrup, puree or extract comprising the following steps: (a) providing a polypeptide of the invention having a xylanase activity, or a xylanase encoded by a nucleic acid of the invention; (b) providing a composition or a liquid comprising a fruit or vegetable material; and (c) contacting the polypeptide of step (a) and the composition, thereby preparing the fruit or vegetable juice, syrup, puree or extract.

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The invention provides papers or paper products or paper pulp comprising a xylanase of the invention, or a polypeptide encoded by a nucleic acid of the invention. The invention provides methods for treating a paper or a paper or wood pulp comprising the following steps: (a) providing a polypeptide of the invention having a xylanase activity, or a xylanase encoded by a nucleic acid of the invention; (b) providing a composition comprising a paper or a paper or wood pulp; and (c) contacting the polypeptide of step (a) and the composition of step (b) under conditions wherein the xylanase can treat the paper or paper or wood pulp. In one aspect, the pharmaceutical composition acts as a digestive aid or an antimicrobial (e.g., against Salmonella). In one aspect, the treatment is prophylactic. In one aspect, the invention provides oral care products comprising a polypeptide of the invention having a xylanase activity, or a xylanase encoded by a nucleic acid of the invention. The oral care product can comprise a toothpaste, a dental cream, a gel or a tooth powder, an odontic, a mouth wash, a pre- or post brushing rinse formulation, a chewing gum, a lozenge or a candy. The invention provides contact lens cleaning compositions comprising a polypeptide of the invention having a xylanase activity, or a xylanase encoded by a nucleic acid of the invention.

In one aspect, the invention provides methods for eliminating or protecting animals from a microorganism comprising a xylan comprising administering a polypeptide of the invention. The microorganism can be a bacterium comprising a xylan, e.g., Salmonella.

The invention provides an isolated nucleic acid having a sequence as set forth in SEQ ID NO.:189 and variants thereof having at least 50% sequence identity to SEQ ID NO.:189 and encoding polypeptides having xylanase activity. In one aspect, the polypeptide has a xylanase activity, e.g., a thermostable xylanase activity.

The invention provides isolated or recombinant nucleic acids comprising SEQ ID NO:189, wherein SEQ ID NO:189 comprises one or more or all of the following sequence variations: the nucleotides at positions 22 to 24 are TTC, the nucleotides at positions 22 to 24

are TTT, the nucleotides at positions 31 to 33 are CAC, the nucleotides at positions 31 to 33 are CAT, the nucleotides at positions 34 to 36 are TTG, the nucleotides at positions 34 to 36 are TTA, the nucleotides at positions 34 to 36 are CTC, the nucleotides at positions 34 to 36 are CTT, the nucleotides at positions 34 to 36 are CTA, the nucleotides at positions 34 to 36 are CTG, the nucleotides at positions 49 to 51 are ATA, the nucleotides at positions 49 to 51 are ATT, the nucleotides at positions 49 to 51 are ATC, the nucleotides at positions 178 to 180 are CAC, the nucleotides at positions 178 to 180 are CAT, the nucleotides at positions 190 to 192 are TGT, the nucleotides at positions 190 to 192 are TGC, the nucleotides at positions 190 to 192 are GTA, the nucleotides at positions 190 to 192 are GTT, the nucleotides at positions 190 to 192 are GTC, the nucleotides at positions 190 to 192 are GTG, the nucleotides at positions 193 to 195 are GTG, the nucleotides at positions 193 to 195 are GTC, the nucleotides at positions 193 to 195 are GTA, the nucleotides at positions 193 to 195 are GTT, the nucleotides at positions 202 to 204 are ATA, the nucleotides at positions 202 to 204 are ATT, the nucleotides at positions 202 to 204 are ATC, the nucleotides at positions 202 to 204 are GCT, the nucleotides at positions 202 to 204 are GCG, the nucleotides at positions 202 to 204 are GCC, the nucleotides at positions 202 to 204 are GCA, the nucleotides at positions 235 to 237 are CCA, the nucleotides at positions 235 to 237 are CCC, or the nucleotides at positions 235 to 237 are CCG.

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The invention provides isolated or recombinant polypeptides comprising an amino acid sequence comprising SEQ ID NO:190, wherein SEQ ID NO:190 comprises one or more or all of the following sequence variations: the aspartic acid at amino acid position 8 is phenylalanine, the glutamine at amino acid position 11 is histidine, the asparagine at amino acid position 12 is leucine, the glycine at amino acid position 17 is isoleucine, the threonine at amino acid position 23 is threonine encoded by a codon other than the wild type codon, the glycine at amino acid position 60 is histidine, the proline at amino acid position 64 is cysteine, the proline at amino acid position 64 is valine, the serine at amino acid position 65 is valine, the glycine at amino acid position 68 is isoleucine, the glycine at amino acid position 68 is alanine, or the serine at amino acid position 79 is proline. In one aspect, the polypeptide has a xylanase activity, e.g., a thermostable xylanase activity.

The invention provides isolated or recombinant nucleic acids comprising SEQ ID NO: 189, wherein SEQ ID NO:189 comprises one or more or all sequence variations set forth in Table 1 or Table 2. The invention provides isolated or recombinant polypeptides encoded by nucleic acids comprising SEQ ID NO: 189, wherein SEQ ID NO:189 comprises

one or more or all sequence variations set forth in Table 1 or Table 2. In one aspect, the polypeptide has a xylanase activity, e.g., a thermostable xylanase activity.

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The invention provides isolated or recombinant nucleic acids comprising SEQ ID NO:379, wherein SEQ ID NO:379 comprises one or more or all of the following sequence variations: the nucleotides at positions 22 to 24 are TTC, the nucleotides at positions 31 to 33 are CAC, the nucleotides at positions 49 to 51 are ATA, the nucleotides at positions 178 to 180 are CAC, the nucleotides at positions 193 to 195 are GTG, the nucleotides at positions 202 to 204 are GCT.

The invention provides isolated or recombinant polypeptides comprising SEQ ID NO:380, wherein SEQ ID NO:380 comprises one or more or all of the following sequence variations: D8F, Q11H, G17I, G60H, S65V and/or G68A. In one aspect, the polypeptide has a xylanase activity, e.g., a thermostable xylanase activity.

The isolated or recombinant nucleic acids of the invention are also referred to as "Group A nucleic acid sequences". The invention provides an isolated nucleic acid including at least 10 consecutive bases of a sequence as set forth in Group A nucleic acid sequences, sequences substantially identical thereto and the sequences complementary thereto.

The isolated or recombinant polypeptides of the invention, which include functional fragments of the exemplary sequences of the invention, are also referred to as "Group B amino acid sequences". Another aspect of the invention is an isolated or recombinant nucleic acid encoding a polypeptide having at least 10 consecutive amino acids of a sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto. In yet another aspect, the invention provides a purified polypeptide having a sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto. Another aspect of the invention is an isolated or purified antibody that specifically binds to a polypeptide having a sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto.

Another aspect of the invention is an isolated or purified antibody or binding fragment thereof, which specifically binds to a polypeptide having at least 10 consecutive amino acids of one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto.

Another aspect of the invention is a method of making a polypeptide having a sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto. The method includes introducing a nucleic acid encoding the polypeptide into a host

cell, wherein the nucleic acid is operably linked to a promoter and culturing the host cell under conditions that allow expression of the nucleic acid. Another aspect of the invention is a method of making a polypeptide having at least 10 amino acids of a sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto. The method includes introducing a nucleic acid encoding the polypeptide into a host cell, wherein the nucleic acid is operably linked to a promoter and culturing the host cell under conditions that allow expression of the nucleic acid, thereby producing the polypeptide.

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Another aspect of the invention is a method of generating a variant including obtaining a nucleic acid having a sequence as set forth in Group A nucleic acid sequences, sequences substantially identical thereto, sequences complementary to the sequences of Group A nucleic acid sequences, fragments comprising at least 30 consecutive nucleotides of the foregoing sequences and changing one or more nucleotides in the sequence to another nucleotide, deleting one or more nucleotides in the sequence, or adding one or more nucleotides to the sequence.

Another aspect of the invention is a computer readable medium having stored thereon a sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto.

Another aspect of the invention is a computer system including a processor and a data storage device wherein the data storage device has stored thereon a sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide having a sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto.

Another aspect of the invention is a method for comparing a first sequence to a reference sequence wherein the first sequence is a nucleic acid having a sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide code of Group B amino acid sequences and sequences substantially identical thereto. The method includes reading the first sequence and the reference sequence through use of a computer program that compares sequences; and determining differences between the first sequence and the reference sequence with the computer program.

Another aspect of the invention is a method for identifying a feature in a sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide having a sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, including reading the sequence through the use of a

computer program which identifies features in sequences; and identifying features in the sequence with the computer program.

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Yet another aspect of the invention is a method of catalyzing the breakdown of xylan or a derivative thereof, comprising the step of contacting a sample containing xylan or the derivative thereof with a polypeptide of Group B amino acid sequences and sequences substantially identical thereto under conditions which facilitate the breakdown of the xylan.

Another aspect of the invention is an assay for identifying fragments or variants of Group B amino acid sequences and sequences substantially identical thereto, which retain the enzymatic function of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto. The assay includes contacting the polypeptide of Group B amino acid sequences, sequences substantially identical thereto, or polypeptide fragment or variant with a substrate molecule under conditions which allow the polypeptide fragment or variant to function and detecting either a decrease in the level of substrate or an increase in the level of the specific reaction product of the reaction between the polypeptide and substrate thereby identifying a fragment or variant of such sequences.

Another aspect of the invention is a nucleic acid probe of an oligonucleotide from about 10 to 50 nucleotides in length and having a segment of at least 10 contiguous nucleotides that is at least 50% complementary to a nucleic acid target region of a nucleic acid sequence selected from the group consisting of Group A nucleic acid sequences; and which hybridizes to the nucleic acid target region under moderate to highly stringent conditions to form a detectable target:probe duplex.

Another aspect of the invention is a polynucleotide probe for isolation or identification of xylanase genes having a sequence which is the same as, or fully complementary to at least a fragment of one of Group A nucleic acid sequences.

In still another aspect, the invention provides a protein preparation comprising a polypeptide having an amino acid sequence selected from Group B amino acid sequences and sequences substantially identical thereto wherein the protein preparation is a liquid.

Still another aspect of the invention provides a protein preparation comprising a polypeptide having an amino acid sequence selected from Group B amino acid sequences and sequences substantially identical thereto wherein the polypeptide is a solid.

Yet another aspect of the invention provides a method for modifying small molecules, comprising the step of mixing at least one polypeptide encoded by a polynucleotide selected from Group A nucleic acid sequences, sequences substantially identical thereto and the sequences complementary thereto with at least one small molecule,

to produce at least one modified small molecule via at least one biocatalytic reaction, where the at least one polypeptide has xylanase activity.

Another aspect of the invention is a cloning vector of a sequence that encodes a polypeptide having xylanase activity, said sequence being selected from Group A nucleic acid sequences, sequences substantially identical thereto and the sequences complementary thereto.

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Another aspect of the invention is a host cell comprising a sequence that encodes a polypeptide having xylanase activity, said sequence being selected from Group A nucleic acid sequences, sequences substantially identical thereto and the sequences complementary thereto.

In yet another aspect, the invention provides an expression vector capable of replicating in a host cell comprising a polynucleotide having a sequence selected Group A nucleic acid sequences, sequences substantially identical thereto, sequences complementary thereto and isolated nucleic acids that hybridize to nucleic acids having any of the foregoing sequences under conditions of low, moderate and high stringency.

In another aspect, the invention provides a method of dough conditioning comprising contacting dough with at least one polypeptide of Group B amino acid sequences and sequences substantially identical thereto under conditions sufficient for conditioning the dough.

Another aspect of the invention is a method of beverage production comprising administration of at least one polypeptide of Group B amino acid sequences and sequences substantially identical thereto under conditions sufficient for decreasing the viscosity of wort or beer.

The xylanases of the invention are used to break down the high molecular weight arabinoxylans in animal feed. Adding the xylanases of the invention stimulates growth rates by improving digestibility, which also improves the quality of the animal litter. Xylanase functions through the gastro-intestinal tract to reduce intestinal viscosity and increase diffusion of pancreatic enzymes. Additionally, the xylanases of the invention may be used in the treatment of endosperm cell walls of feed grains and vegetable proteins. In one aspect of the invention, the novel xylanases of the invention are administered to an animal in order to increase the utilization of the xylan in the food. This activity of the xylanases of the invention may be used to break down insoluble cell wall material, liberating nutrients in the cell walls, which then become available to the animal. It also changes hemicellulose to

nutritive sugars so that nutrients formerly trapped within the cell walls are released.

Xylanase also produces compounds that may be a nutritive source for the ruminal microflora.

Another aspect of the invention provides a method for utilizing xylanase as a nutritional supplement in the diets of animals, comprising preparation of a nutritional supplement containing a recombinant xylanase enzyme comprising at least thirty contiguous amino acids of Group B amino acid sequences and sequences substantially identical thereto and administering the nutritional supplement to an animal to increase the utilization of xylan contained in food ingested by the animal.

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In another aspect of the invention, a method for delivering a xylanase supplement to an animal is provided, where the method comprises preparing an edible enzyme delivery matrix in the form of pellets comprising a granulate edible carrier and a thermostable recombinant xylanase enzyme, wherein the particles readily disperse the xylanase enzyme contained therein into aqueous media, and administering the edible enzyme delivery matrix to the animal. The granulate edible carrier may comprise a carrier selected from the group consisting of grain germ that is spent of oil, hay, alfalfa, timothy, soy hull, sunflower seed meal and wheat midd. The xylanase enzyme may have an amino acid sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto.

In another aspect, the invention provides an isolated nucleic acid comprising a

sequence that encodes a polypeptide having xylanase activity, wherein the sequence is
selected from Group A nucleic acid sequences, sequences substantially identical thereto and
the sequences complementary thereto, wherein the sequence contains a signal sequence. The
invention also provides an isolated nucleic acid comprising a sequence that encodes a
polypeptide having xylanase activity, wherein the sequence is selected from Group A nucleic
acid sequences, sequences substantially identical thereto and the sequences complementary
thereto, wherein the sequence contains a signal sequence from another xylanase.
Additionally, the invention provides an isolated nucleic acid comprising a sequence that
encodes a polypeptide having xylanase activity, wherein the sequence is selected from Group
A nucleic acid sequences, sequences substantially identical thereto and the sequences
complementary thereto wherein the sequence does not contain a signal sequence.

Still another aspect of the invention provides an isolated nucleic acid that is a mutation of SEQ ID NO: 189. Yet another aspect provides an amino acid sequence that is a mutation of SEQ ID NO: 190.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

All publications, patents, patent applications, GenBank sequences and ATCC deposits, cited herein are hereby expressly incorporated by reference for all purposes.

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BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are illustrative of aspects of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

The patent or application file contains at least one drawing executed in color.

Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 is a block diagram of a computer system.

Figure 2 is a flow diagram illustrating one aspect of a process for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database.

Figure 3 is a flow diagram illustrating one aspect of a process in a computer for determining whether two sequences are homologous.

Figure 4 is a flow diagram illustrating one aspect of an identifier process 300 for detecting the presence of a feature in a sequence.

Figure 5 is a graph comparing activity of the wild type sequence (SEQ ID NOS: 189 and 190) to the 8x mutant (SEQ ID NOS:375, 376), a combination of mutants D, F, H, I, S, V, X and AA in Table 1.

Figure 6A illustrates the nine single site amino acid mutants of SEQ ID NO:378 (encoded by SEQ ID NO:377) as generated by Gene Site Saturation Mutagenesis (GSSMTM) of SEQ ID NO:190 (encoded by SEQ ID NO:189), as described in detail in Example 5, below.

Figure 6B illustrates the unfolding of SEQ ID NO:190 and SEQ ID NO:378 in melting temperature transition midpoint (Tm) experiments as determined by DSC for each enzyme, as described in detail in Example 5, below.

Figure 7A illustrates the pH and temperature activity profiles for the enzymes SEQ ID NO:190 and SEQ ID NO:378, as described in detail in Example 5, below.

Figure 7B illustrates the rate/temperature activity optima for the enzymes SEQ ID NO:190 and SEQ ID NO:378, as described in detail in Example 5, below.

Figure 7C illustrates the thermal tolerance/ residual activity for the enzymes SEO ID NO:190 and SEO ID NO:378, as described in detail in Example 5, below.

Figure 8A illustrates the GeneReassembly™ library of all possible combinations of the 9 GSSM™ point mutations that was constructed and screened for variants with improved thermal tolerance and activity, as described in detail in Example 5, below.

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Figure 8B illustrates the relative activity of the "6X-2" variant and "9X" variant (SEQ ID NO:378) compared to SEQ ID NO:190 ("wild-type") at a temperature optimum and pH 6.0, as described in detail in Example 5, below.

Figure 9A illustrates the fingerprints obtained after hydrolysis of oligoxylans (Xyl)3, (Xyl)4, (Xyl)5 and (Xyl)6 by the SEQ ID NO:190 ("wild-type") and the "9X" variant (SEQ ID NO:378) enzymes, as described in detail in Example 5, below.

Figure 9B illustrates the fingerprints obtained after hydrolysis of Beechwood xylan by the SEQ ID NO:190 ("wild-type") and the "9X" variant (SEQ ID NO:378) enzymes, as described in detail in Example 5, below.

Figure 10A is a schematic diagram illustrating the level of thermal stability (represented by Tm) improvement over SEQ ID NO:190 ("wild-type") obtained by GSSMTM evolution, as described in detail in Example 5, below.

Figure 10B illustrates a "fitness diagram" of enzyme improvement in the form of SEQ ID NO:378 and SEQ ID NO:380, as obtained by combining GSSMTM and GeneReassemblyTM technologies, as described in detail in Example 5, below.

Figure 11 is a schematic flow diagram of an exemplary routine screening protocol to determine whether a xylanase of the invention is useful in pretreating paper pulp, as described in detail in Example 6, below.

Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to xylanases and polynucleotides encoding them and methods of making and using them. Xylanase activity of the polypeptides of the invention encompasses enzymes having hydrolase activity, for example, enzymes capable of hydrolyzing glycosidic linkages present in xylan, e.g., catalyzing hydrolysis of internal β -1,4-xylosidic linkages. The xylanases of the invention can be used to make and/or process foods, feeds, nutritional supplements, textiles, detergents and the like. The xylanases of the

invention can be used in pharmaceutical compositions and dietary aids. Xylanases of the invention are particularly useful in baking, animal feed, beverage and paper processes.

Definitions

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The term "antibody" includes a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope, see, e.g.

Fundamental Immunology, Third Edition, W.E. Paul, ed., Raven Press, N.Y. (1993); Wilson (1994) J. Immunol. Methods 175:267-273; Yarmush (1992) J. Biochem. Biophys. Methods 25:85-97. The term antibody includes antigen-binding portions, i.e., "antigen binding sites," (e.g., fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term "antibody."

The terms "array" or "microarray" or "biochip" or "chip" as used herein is a plurality of target elements, each target element comprising a defined amount of one or more polypeptides (including antibodies) or nucleic acids immobilized onto a defined area of a substrate surface, as discussed in further detail, below.

As used herein, the terms "computer," "computer program" and "processor" are used in their broadest general contexts and incorporate all such devices, as described in detail, below. A "coding sequence of" or a "sequence encodes" a particular polypeptide or protein, is a nucleic acid sequence which is transcribed and translated into a polypeptide or protein when placed under the control of appropriate regulatory sequences.

The phrases "nucleic acid" or "nucleic acid sequence" as used herein refer to an oligonucleotide, nucleotide, polynucleotide, or to a fragment of any of these, to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent a sense or antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material, natural or synthetic in origin. The phrases "nucleic acid" or "nucleic acid sequence" includes oligonucleotide, nucleotide, polynucleotide, or to a fragment of any of these, to DNA or RNA (e.g., mRNA, rRNA, tRNA, iRNA) of genomic or synthetic origin

which may be single-stranded or double-stranded and may represent a sense or antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material, natural or synthetic in origin, including, e.g., iRNA, ribonucleoproteins (e.g., e.g., double stranded iRNAs, e.g., iRNPs). The term encompasses nucleic acids, i.e., oligonucleotides, containing known analogues of natural nucleotides. The term also encompasses nucleic-acid-like structures with synthetic backbones, see e.g., Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197; Strauss-Soukup (1997) Biochemistry 36:8692-8698; Samstag (1996) Antisense Nucleic Acid Drug Dev 6:153-156. "Oligonucleotide" includes either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands that may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide can ligate to a fragment that has not been dephosphorylated.

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A "coding sequence of" or a "nucleotide sequence encoding" a particular polypeptide or protein, is a nucleic acid sequence which is transcribed and translated into a polypeptide or protein when placed under the control of appropriate regulatory sequences.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as, where applicable, intervening sequences (introns) between individual coding segments (exons). "Operably linked" as used herein refers to a functional relationship between two or more nucleic acid (e.g., DNA) segments. Typically, it refers to the functional relationship of transcriptional regulatory sequence to a transcribed sequence. For example, a promoter is operably linked to a coding sequence, such as a nucleic acid of the invention, if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, i.e., they are cis-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

The term "expression cassette" as used herein refers to a nucleotide sequence which is capable of affecting expression of a structural gene (i.e., a protein coding sequence, such as a xylanase of the invention) in a host compatible with such sequences. Expression cassettes include at least a promoter operably linked with the polypeptide coding sequence; and, optionally, with other sequences, e.g., transcription termination signals. Additional

factors necessary or helpful in effecting expression may also be used, e.g., enhancers. Thus, expression cassettes also include plasmids, expression vectors, recombinant viruses, any form of recombinant "naked DNA" vector, and the like. A "vector" comprises a nucleic acid that can infect, transfect, transiently or permanently transduce a cell. It will be recognized that a vector can be a naked nucleic acid, or a nucleic acid complexed with protein or lipid. The vector optionally comprises viral or bacterial nucleic acids and/or proteins, and/or membranes (e.g., a cell membrane, a viral lipid envelope, etc.). Vectors include, but are not limited to replicons (e.g., RNA replicons, bacteriophages) to which fragments of DNA may be attached and become replicated. Vectors thus include, but are not limited to RNA, autonomous selfreplicating circular or linear DNA or RNA (e.g., plasmids, viruses, and the like, see, e.g., U.S. Patent No. 5,217,879), and include both the expression and non-expression plasmids. Where a recombinant microorganism or cell culture is described as hosting an "expression vector" this includes both extra-chromosomal circular and linear DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or is incorporated within the host's genome.

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As used herein, the term "promoter" includes all sequences capable of driving transcription of a coding sequence in a cell, e.g., a plant cell. Thus, promoters used in the constructs of the invention include *cis*-acting transcriptional control elements and regulatory sequences that are involved in regulating or modulating the timing and/or rate of transcription of a gene. For example, a promoter can be a *cis*-acting transcriptional control element, including an enhancer, a promoter, a transcription terminator, an origin of replication, a chromosomal integration sequence, 5' and 3' untranslated regions, or an intronic sequence, which are involved in transcriptional regulation. These cis-acting sequences typically interact with proteins or other biomolecules to carry out (turn on/off, regulate, modulate, etc.) transcription. "Constitutive" promoters are those that drive expression continuously under most environmental conditions and states of development or cell differentiation. "Inducible" or "regulatable" promoters direct expression of the nucleic acid of the invention under the influence of environmental conditions or developmental conditions. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, drought, or the presence of light.

"Tissue-specific" promoters are transcriptional control elements that are only active in particular cells or tissues or organs, e.g., in plants or animals. Tissue-specific regulation may be achieved by certain intrinsic factors that ensure that genes encoding

proteins specific to a given tissue are expressed. Such factors are known to exist in mammals and plants so as to allow for specific tissues to develop.

The term "plant" includes whole plants, plant parts (e.g., leaves, stems, flowers, roots, etc.), plant protoplasts, seeds and plant cells and progeny of same. The class of plants which can be used in the method of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including angiosperms (monocotyledonous and dicotyledonous plants), as well as gymnosperms. It includes plants of a variety of ploidy levels, including polyploid, diploid, haploid and hemizygous states. As used herein, the term "transgenic plant" includes plants or plant cells into which a heterologous nucleic acid sequence has been inserted, e.g., the nucleic acids and various recombinant constructs (e.g., expression cassettes) of the invention.

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"Plasmids" can be commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. Equivalent plasmids to those described herein are known in the art and will be apparent to the ordinarily skilled artisan.

"Amino acid" or "amino acid sequence" as used herein refer to an oligopeptide, peptide, polypeptide, or protein sequence, or to a fragment, portion, or subunit of any of these and to naturally occurring or synthetic molecules.

"Amino acid" or "amino acid sequence" include an oligopeptide, peptide, polypeptide, or protein sequence, or to a fragment, portion, or subunit of any of these, and to naturally occurring or synthetic molecules. The term "polypeptide" as used herein, refers to amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres and may contain modified amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also a given polypeptide may have many types of modifications. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphytidylinositol, cross-linking cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of

pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristolyation, oxidation, pegylation, xylan hydrolase processing, phosphorylation, prenylation, racemization, selenoylation, sulfation and transfer-RNA mediated addition of amino acids to protein such as arginylation. (See Creighton, T.E., Proteins – Structure and Molecular Properties 2nd Ed., W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B.C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983)). The peptides and polypeptides of the invention also include all "mimetic" and "peptidomimetic" forms, as described in further detail, below.

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As used herein, the term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition and still be isolated in that such vector or composition is not part of its natural environment. As used herein, the term "purified" does not require absolute purity; rather, it is intended as a relative definition. Individual nucleic acids obtained from a library have been conventionally purified to electrophoretic homogeneity. The sequences obtained from these clones could not be obtained directly either from the library or from total human DNA. The purified nucleic acids of the invention have been purified from the remainder of the genomic DNA in the organism by at least 10⁴-10⁶ fold. However, the term "purified" also includes nucleic acids that have been purified from the remainder of the genomic DNA or from other sequences in a library or other environment by at least one order of magnitude, typically two or three orders and more typically four or five orders of magnitude.

As used herein, the term "recombinant" means that the nucleic acid is adjacent to a "backbone" nucleic acid to which it is not adjacent in its natural environment. Additionally, to be "enriched" the nucleic acids will represent 5% or more of the number of nucleic acid inserts in a population of nucleic acid backbone molecules. Backbone molecules according to the invention include nucleic acids such as expression vectors, self-replicating nucleic acids, viruses, integrating nucleic acids and other vectors or nucleic acids used to maintain or manipulate a nucleic acid insert of interest. Typically, the enriched nucleic acids represent 15% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules. More typically, the enriched nucleic acids represent 50% or more of the number of nucleic acid inserts

in the population of recombinant backbone molecules. In a one aspect, the enriched nucleic acids represent 90% or more of the number of nucleic acid inserts in the population of recombinant backbone molecules.

"Recombinant" polypeptides or proteins refer to polypeptides or proteins 5 produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide or protein. "Synthetic" polypeptides or protein are those prepared by chemical synthesis. Solid-phase chemical peptide synthesis methods can also be used to synthesize the polypeptide or fragments of the invention. Such method have been known in the art since the early 1960's (Merrifield, R. B., J. 10 Am. Chem. Soc., 85:2149-2154, 1963) (See also Stewart, J. M. and Young, J. D., Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Co., Rockford, Ill., pp. 11-12)) and have recently been employed in commercially available laboratory peptide design and synthesis kits (Cambridge Research Biochemicals). Such commercially available laboratory kits have generally utilized the teachings of H. M. Geysen et al, Proc. Natl. Acad. Sci., USA, 81:3998 (1984) and provide for synthesizing peptides upon the tips of a multitude of "rods" or "pins" all 15 of which are connected to a single plate. When such a system is utilized, a plate of rods or pins is inverted and inserted into a second plate of corresponding wells or reservoirs, which contain solutions for attaching or anchoring an appropriate amino acid to the pin's or rod's tips. By repeating such a process step, i.e., inverting and inserting the rod's and pin's tips into appropriate 20 solutions, amino acids are built into desired peptides. In addition, a number of available FMOC peptide synthesis systems are available. For example, assembly of a polypeptide or fragment can be carried out on a solid support using an Applied Biosystems, Inc. Model 431A automated peptide synthesizer. Such equipment provides ready access to the peptides of the invention, either by direct synthesis or by synthesis of a series of fragments that can be coupled using 25 other known techniques.

A promoter sequence is "operably linked to" a coding sequence when RNA polymerase which initiates transcription at the promoter will transcribe the coding sequence into mRNA.

"Plasmids" are designated by a lower case "p" preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described herein are known in the art and will be apparent to the ordinarily skilled artisan.

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"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion, gel electrophoresis may be performed to isolate the desired fragment.

The phrase "substantially identical" in the context of two nucleic acids or polypeptides, refers to two or more sequences that have, e.g., at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more nucleotide or amino acid residue (sequence) identity, when compared and aligned for maximum correspondence, as measured using one of the known sequence comparison algorithms or by visual inspection. Typically, the substantial identity exists over a region of at least about 100 residues and most commonly the sequences are substantially identical over at least about 150-200 residues. In some aspects, the sequences are substantially identical over the entire length of the coding regions.

Additionally a "substantially identical" amino acid sequence is a sequence that differs from a reference sequence by one or more conservative or non-conservative amino acid substitutions, deletions, or insertions, particularly when such a substitution occurs at a site that is not the active site of the molecule and provided that the polypeptide essentially retains its functional properties. A conservative amino acid substitution, for example, substitutes one amino acid for another of the same class (e.g., substitution of one hydrophobic amino acid, such as isoleucine, valine, leucine, or methionine, for another, or substitution of one polar amino acid for another, such as substitution of arginine for lysine, glutamic acid for aspartic acid or glutamine for asparagine). One or more amino acids can be deleted, for example, from a xylanase polypeptide, resulting in modification of the structure of the polypeptide, without significantly altering its biological activity. For example, amino- or

carboxyl-terminal amino acids that are not required for xylanase biological activity can be removed. Modified polypeptide sequences of the invention can be assayed for xylanase biological activity by any number of methods, including contacting the modified polypeptide sequence with a xylanase substrate and determining whether the modified polypeptide decreases the amount of specific substrate in the assay or increases the bioproducts of the enzymatic reaction of a functional xylanase polypeptide with the substrate.

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"Fragments" as used herein are a portion of a naturally occurring protein which can exist in at least two different conformations. Fragments can have the same or substantially the same amino acid sequence as the naturally occurring protein. "Substantially the same" means that an amino acid sequence is largely, but not entirely, the same, but retains at least one functional activity of the sequence to which it is related. In general two amino acid sequences are "substantially the same" or "substantially homologous" if they are at least about 85% identical. Fragments which have different three dimensional structures as the naturally occurring protein are also included. An example of this, is a "pro-form" molecule, such as a low activity proprotein that can be modified by cleavage to produce a mature enzyme with significantly higher activity.

"Hybridization" refers to the process by which a nucleic acid strand joins with a complementary strand through base pairing. Hybridization reactions can be sensitive and selective so that a particular sequence of interest can be identified even in samples in which it is present at low concentrations. Suitably stringent conditions can be defined by, for example, the concentrations of salt or formamide in the prehybridization and hybridization solutions, or by the hybridization temperature and are well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature. In alternative aspects, nucleic acids of the invention are defined by their ability to hybridize under various stringency conditions (e.g., high, medium, and low), as set forth herein.

For example, hybridization under high stringency conditions could occur in about 50% formamide at about 37°C to 42°C. Hybridization could occur under reduced stringency conditions in about 35% to 25% formamide at about 30°C to 35°C. In particular, hybridization could occur under high stringency conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS and 200 n/ml sheared and denatured salmon sperm DNA. Hybridization could occur under reduced stringency conditions as described above, but in 35% formamide at a reduced temperature of 35°C. The temperature range corresponding to a particular level of stringency can be further narrowed by calculating the purine to pyrimidine ratio of the

nucleic acid of interest and adjusting the temperature accordingly. Variations on the above ranges and conditions are well known in the art.

The term "variant" refers to polynucleotides or polypeptides of the invention modified at one or more base pairs, codons, introns, exons, or amino acid residues (respectively) yet still retain the biological activity of a xylanase of the invention. Variants can be produced by any number of means included methods such as, for example, error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly (e.g., GeneReassemblyTM, see, e.g., U.S. Patent No. 6,537,776), GSSMTM and any combination thereof.

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Table 1 and Table 2 list variants obtained by mutating SEQ ID NO:189 (encoding SEQ ID NO:190) by GSSM™. The invention provides nucleic acids having one or more, or all, of the sequences as set forth in Tables 1 and 2, i.e., nucleic acids having sequences that are variants of SEQ ID NO:189, where the variations are set forth in Table 1 and Table 2, and the polypeptides that are encoded by these variants.

These GSSMTM variants (set forth in Tables 1 and 2) were tested for thermal tolerance (see Examples, below). Mutants D, F, G, H, I, J, K, S, T, U, V, W, X, Y, Z, AA, DD and EE were found to have the highest thermal tolerance among the mutants in Table 1. Mutants may also be combined to form a larger mutant. For example, mutants D, F, H, I, S, V, X and AA of Table 1 were combined to form a larger mutant termed "8x" with a sequence as set forth in SEQ ID NO:375 (polypeptide encoding nucleic acid) and SEQ ID NO:376 (amino acid sequence). Figure 5 is a graph comparing the activity of the wild type sequence (SEQ ID NOS: 189 and 190) to the 8x mutant (SEQ ID NOS: 259 and 260). In comparing the wild type and the 8x mutant, it was discovered that the optimal temperature for both was 65°C and that the optimal pH for both was 5.5. The wild type sequence was found to maintain its stability for less than 1 minute at 65°C, while the 8x mutant (SEQ ID NOS:375, 376) was found to maintain its stability for more than 10 minutes at 85°C. The substrate used was AZO-AZO-xylan. In one aspect, the 8x mutant (SEQ ID NOS:375, 376) was evolved by GSSMTM. In another aspect, the wild type is a GSSMTM parent for thermal tolerance evolution.

Table 1

Mutant	Mutation	Wild type Seq	GSSM™ Seq
A	A2F	GCC	TIT
B C D E	A2D	GCC	GAC
С	A5H	GCT	CAC
D	D8F	GAC	TTC
E	Q11L	CAA	СТС
F	Q11H	CAA	CAC
G	N12L	AAT	TTG
Н	N12L	AAT	TTG
I	G17I	GGT	ATA
J	Q11H,T23T	CAA,ACC	CAT,ACG
K	Q11H	CAA	CAT
L	S26P	TCT	CCG
М	S26P	TCT	CCA
N	S35F	TCA	ПТ
0	No Change	GTT	GTA
Р	A51P	GCA	CCG
Q_	A51P	GCA	CCG
R	G60R	GGA	CGC
S T	G60H	GGA	CAC
T	G60H	GGA	CAC
U	P64C	CCG	TGT
V	P64V	CCG	GTA
W	P64V	CCG	GTT
X Y	S65V	TCC	GTG
Y	Q11H	CAA	CAT
Z	G68I	GGA	ATA
AA	G68A	GGA	GCT
BB	A71G	GCT	GGA
CC	No Change	AAT	AAC
DD	S79P	TCA	CCA
EE	S79P	TCA	CCC
FF	T95S	ACT	TCT
GG	Y98P	TAT	CCG
НН	T114S	ACT	AGC
II	No Change	AAC	AAC
JJ	No Change	AGG	AGA
KK	I142L	ATT	CTG
LL	S151I	AGC	ATC
MM	S138T,S151A	TCG,AGC	ACG,GCG
NN	K158R	AAG	CGG
00	K160V,V172I	AAA,GTA	GTT,ATA

The codon variants as set forth in Table 2 that produced variants (of SEQ ID NO:189) with the best variation or "improvement" over "wild type" (SEQ ID NO:189) in thermal tolerance are highlighted. As noted above, the invention provides nucleic acids, and the polypeptides that encode them, comprising one, several or all or the variations set forth in Table 2 and Table 1.

Table 2

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Mutation	Wild type Sequence	GSSM™ Sequence	Other codons also coding for same changed amino acid
A2F	GCC	TTT	ттс
A2D	GCC	GAC	GAT
A5H	GCT	CAC	CAT
D8 1	GAC	SPEC	TTT
Q11L	CAA	CTC	TTA, TTG, CTT, CTA, CTG
可能增	CAA	CAG, CAT	-
NEZI	AAT	TITE	TTA, CTC, CTT, CTA, CTG
Ci M	GGT	ATEN	ATT, ATC
T23T	ACC	ACG	ACT, ACC, ACA
S26P	TCT	CCG, CCA	CCC
S35F	TCA	TTT	TTC
A51P	GCA	CCG	CCC, CCA
G60R	GGA	CGC	CGT, CGA, CGG, AGA, AGG
SIS(0)H	GGA	EXX	CAT
Pi02(0	CCG	iieii	TGC
25.	CCG	cia, ci	GTC, GTG
SPER	TCC	EIF	GTC, GTA, GTT
<u>इहिंही</u>	GGA	AT A	ATT, ATC
268 4	GGA	e (e) i	GCG, GCC, GCA
A71G	GCT	GGA	GGT, GGC, GGG
S76912	TCA	COA, DOG	cce ,
T95S	ACT	TCT	TCC, TCA, TCG, AGT, AGC
Y98P	TAT	CCG	CCC, CCA
T114S	ACT	AGC	TCC, TCA, TCG, AGT, TCT
1142L	ATT	CTG	TTA, CTC, CTT, CTA, TTG
S151I	AGC	ATC	ATT, ATA
S138T	TCG	ACG	ACT, ACC, ACA
S151A	AGC	GCG	GCT, GCC, GCA
K158R	AAG	CGG	CGT, CGA, CGC, AGA, AGG
K160V	AAA	GTT	GTC, GTA, GTG
V172I	GTA	ATA	ATT, ATC

In one aspect the amino acid sequence of an amino acid sequence (SEQ ID NO: 208) of Group B amino acid sequences is modified by a single amino acid mutation. In a specific aspect, that mutation is an asparagine to aspartic acid mutation. The resulting amino acid sequence and corresponding nucleic acid sequence are set forth as SEQ ID NO:252 and SEQ ID NO:251, respectively. Single amino acid mutations with an improvement in the pH optimum of the enzyme, such as the mutation of SEQ ID NO:208, have been shown in the art with respect to xylanases. (See, for example, Joshi, M., Sidhu, G., Pot, I., Brayer, G., Withers, S., McIntosh, L., J. Mol. Bio. 299, 255-279 (2000).) It is also noted that in such single amino acid mutations, portions of the sequences may be removed in the subcloning process. For example, SEQ ID NO:207 and SEQ ID NO:251 differ in only

one nucleotide, over the area that the sequences align. However, it is noted that a 78 nucleotide area at the N-terminus of SEQ ID NO:207 was removed from the N-terminus of SEQ ID NO:251 in the subcloning. Additionally, the first three nucleotides in SEQ ID NO:251 were changed to ATG and then the point mutation was made at the sixth nucleotide in SEQ ID NO:251.

The term "saturation mutagenesis", "gene site saturated mutagenesis" or "GSSMTM" includes a method that uses degenerate oligonucleotide primers to introduce point mutations into a polynucleotide, as described in detail, below.

The term "optimized directed evolution system" or "optimized directed evolution" includes a method for reassembling fragments of related nucleic acid sequences, e.g., related genes, and explained in detail, below.

The term "synthetic ligation reassembly" or "SLR" includes a method of ligating oligonucleotide fragments in a non-stochastic fashion, and explained in detail, below.

Generating and Manipulating Nucleic Acids

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The invention provides nucleic acids (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEO ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEO ID NO:73, SEO ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEO ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEO ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEO ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193,

SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, 5 SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, 10 SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, 15 SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379; nucleic acids encoding polypeptides as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, 20 SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, S NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, S NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:64, SEQ ID NO:64, SEQ ID NO:65, S 25 NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, S NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, 30 SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132; SEQ ID NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; SEQ ID NO:142; SEQ ID NO:144; NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158, SEQ ID

NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID 5 NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, SEQ ID 10 NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278, SEQ ID NO:280, SEQ ID NO:282, SEQ ID NO:284, SEQ ID NO:286, SEQ ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID 15 NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, SEQ ID NO:320, SEQ ID NO:322, SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID NO:334, SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID 20 NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376, SEQ ID NO:378 or SEQ ID NO:380), including expression cassettes such as expression vectors, encoding the polypeptides of the invention. The invention also includes methods for discovering new xylanase sequences using the nucleic acids of the invention. The invention also includes 25 methods for inhibiting the expression of xylanase genes, transcripts and polypeptides using the nucleic acids of the invention. Also provided are methods for modifying the nucleic acids of the invention by, e.g., synthetic ligation reassembly, optimized directed evolution system and/or saturation mutagenesis.

The nucleic acids of the invention can be made, isolated and/or manipulated by, e.g., cloning and expression of cDNA libraries, amplification of message or genomic DNA by PCR, and the like. For example, the following exemplary sequences of the invention were initially derived from the following sources:

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Table 3

	SEQ ID	SOURCE
	1, 2	Bacteria
	101, 102	Environmental
5	103, 104	Bacteria
	105, 106	Environmental
	107, 108	Bacteria
	109, 110	Environmental
	11, 12	Environmental
10	111, 112	Environmental
10	113, 114	Environmental
	115, 116	Environmental
	117, 118	Environmental
	119, 120	Environmental
15	121, 122	Environmental
13	123, 124	Environmental
	125, 124	Environmental
	127, 128	Environmental
	129, 130	Bacteria
20	13, 14	Environmental
20	131, 132	Environmental
	133, 134	Environmental
	135, 134	Environmental
	137, 138	Environmental
25	139, 140	Environmental
23	141, 142	Environmental
	141, 142	Bacteria
	145, 144	Eukaryote
	143, 146	Environmental
20	147, 148	Environmental
30		
	15, 16	Environmental
	151, 152	Environmental Environmental
	153, 154	
25	155, 156 157, 158	Environmental
35	157, 156	Environmental
	159, 160 161, 162	Environmental
		Environmental
	163, 164	Environmental
40	165, 166 167, 168	Environmental
40	167, 108	Environmental
	169, 170	Environmental
	17, 18	Bacteria
	171, 172	Environmental
	173, 174	Environmental
45	175, 176	Environmental
	177, 178	Environmental
	179, 180	Environmental
	181, 182	Environmental
	183, 184	Environmental
50	185, 186	Environmental

	187, 188	Environmental
	189, 190	Environmental
	19, 20	Environmental
	191, 192	Environmental
5	193, 194	Environmental
	195, 196	Environmental
	195, 196 197, 198	Environmental
	199, 200	Environmental
	201, 202	Environmental Environmental
10		Environmental
10	203, 204	Environmental
	205, 206	
	207, 208	Environmental
	209, 210	Environmental
	21, 22	Environmental
15	211, 212	Environmental
	213, 214	Environmental
	215, 216	Environmental
	217, 218	Environmental
	219, 220	Environmental
20	221, 222	Environmental
	223, 224	Environmental
	225, 226	Environmental
	227, 228	Environmental
	229, 230	Environmental
25	23, 24	Environmental
	231, 232	Bacteria
	233, 234	Environmental
	235, 236	Environmental
	237, 238	Environmental
30	239, 240	Environmental
	241, 242	Environmental
	243, 244	Environmental
	245, 246	Environmental
	247, 248	Environmental
35	249, 250	Environmental
33	25, 26	Environmental
	251, 252	Environmental
	253, 254	Environmental Environmental
		Environmental
40	255, 256 257, 258	Environmental
40	257, 258	
	259, 260	Environmental
	261, 262	Environmental
	263, 264	Environmental
	265, 266	Environmental
45	267, 268	Bacteria
	269, 270	Environmental
	27, 28	Environmental
	271, 272	Environmental
	273, 274	Environmental
50	275, 276	Environmental

	277, 278	Environmental
	279, 280	Environmental
	281, 282	Environmental
	283, 284	Environmental
5	285, 286	Environmental
	287, 288	Environmental
	289, 290	Environmental
	29, 30	Archaea
	291, 292	Environmental
10	293, 294	Environmental
	295, 296	Environmental
	297, 298	Environmental
	299, 300	Environmental
	3, 4	Environmental
15	301, 302	Environmental
	303, 304	Environmental
	305, 306	Bacteria
	307, 308	Environmental
	309, 310	Environmental
20	31, 32	Environmental
	311, 312	Environmental
	313, 314	Bacteria
	315, 316	Environmental
	317, 318	Environmental
25	319, 320	Environmental
	321, 322	Environmental
	323, 324	Environmental
	325, 326	Environmental
	327, 328	Environmental
30	329, 330	Environmental
_	33, 34	Environmental
	331, 332	Environmental
	333, 334	Environmental
	335, 336	Environmental
35	337, 338	Environmental
	339, 340	Environmental
	341, 342	Environmental
	343, 344	Environmental
	345, 346	Environmental
40	347, 348	Environmental
-	349, 350	Environmental
	35, 36	Environmental
	351, 352	Environmental
	353, 354	Environmental
45	355, 356	Environmental
**	357, 358	Environmental
	359, 360	Environmental
	361, 362	Environmental
	363, 364	Environmental
50	365, 366	Environmental
- -	,	

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367, 368	Environmental	
369, 370	Environmental	
37, 38	Environmental	
371, 372	Environmental	

	369, 370	Environmental
	37, 38	Environmental
	371, 372	Environmental
5	371, 372 373, 374	Environmental
	375, 376	Artificial
	377, 378	Artificial
	39, 40	Environmental
	41, 42	Environmental
10	43, 44	Environmental
	45, 46	Environmental
	47, 48	Environmental
	49, 50	Environmental
	5,6	Environmental
15	51, 52	Environmental
	53, 54	Bacteria
	55, 56	Environmental
	57, 58	Environmental
	59, 60	Environmental
20	61, 62	Environmental
	63, 64	Environmental
	65, 66	Environmental
	67, 68	Environmental
	69, 70	Environmental
25	7,8	Environmental
	71, 72	Environmental
	73, 74	Environmental
	75, 76	Environmental
	77, 78	Environmental
30	79, 80	Environmental
	81, 82	Environmental
	83, 84	Environmental
	85, 86	Bacteria
	87, 88	Environmental
35	89, 90	Bacteria
	9, 10	Environmental
	91, 92	Environmental
	93, 94	Environmental
40	95, 96	Environmental
40	97, 98	Environmental
	99, 100	Environmental

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In one aspect, the invention also provides xylanase-encoding nucleic acids with a common novelty in that they are derived from an environmental source, or a bacterial source, or an archaeal source.

In practicing the methods of the invention, homologous genes can be modified by manipulating a template nucleic acid, as described herein. The invention can be practiced

in conjunction with any method or protocol or device known in the art, which are well described in the scientific and patent literature.

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One aspect of the invention is an isolated nucleic acid comprising one of the sequences of Group A nucleic acid sequences and sequences substantially identical thereto, the sequences complementary thereto, or a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive bases of one of the sequences of a Group A nucleic acid sequence (or the sequences complementary thereto). The isolated, nucleic acids may comprise DNA, including cDNA, genomic DNA and synthetic DNA. The DNA may be double-stranded or single-stranded and if single stranded may be the coding strand or non-coding (anti-sense) strand. Alternatively, the isolated nucleic acids may comprise RNA.

As discussed in more detail below, the isolated nucleic acids of one of the Group A nucleic acid sequences and sequences substantially identical thereto, may be used to prepare one of the polypeptides of a Group B amino acid sequence and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto.

Accordingly, another aspect of the invention is an isolated nucleic acid which encodes one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of one of the polypeptides of the Group B amino acid sequences. The coding sequences of these nucleic acids may be identical to one of the coding sequences of one of the nucleic acids of Group A nucleic acid sequences, or a fragment thereof or may be different coding sequences which encode one of the polypeptides of Group B amino acid sequences, sequences substantially identical thereto and fragments having at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of one of the polypeptides of Group B amino acid sequences, as a result of the redundancy or degeneracy of the genetic code. The genetic code is well known to those of skill in the art and can be obtained, for example, on page 214 of B. Lewin, Genes VI, Oxford University Press, 1997.

The isolated nucleic acid which encodes one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, may include, but is not limited to: only the coding sequence of one of Group A nucleic acid sequences and sequences substantially identical thereto and additional coding sequences, such as leader sequences or

proprotein sequences and non-coding sequences, such as introns or non-coding sequences 5' and/or 3' of the coding sequence. Thus, as used herein, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only the coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

Alternatively, the nucleic acid sequences of Group A nucleic acid sequences and sequences substantially identical thereto, may be mutagenized using conventional techniques, such as site directed mutagenesis, or other techniques familiar to those skilled in the art, to introduce silent changes into the polynucleotides of Group A nucleic acid sequences and sequences substantially identical thereto. As used herein, "silent changes" include, for example, changes which do not alter the amino acid sequence encoded by the polynucleotide. Such changes may be desirable in order to increase the level of the polypeptide produced by host cells containing a vector encoding the polypeptide by introducing codons or codon pairs which occur frequently in the host organism.

The invention also relates to polynucleotides which have nucleotide changes which result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptides of Group B amino acid sequences and sequences substantially identical thereto. Such nucleotide changes may be introduced using techniques such as site directed mutagenesis, random chemical mutagenesis, exonuclease III deletion and other recombinant DNA techniques. Alternatively, such nucleotide changes may be naturally occurring allelic variants which are isolated by identifying nucleic acids which specifically hybridize to probes comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive bases of one of the sequences of Group A nucleic acid sequences and sequences substantially identical thereto (or the sequences complementary thereto) under conditions of high, moderate, or low stringency as provided herein.

General Techniques

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The nucleic acids used to practice this invention, whether RNA, iRNA, antisense nucleic acid, cDNA, genomic DNA, vectors, viruses or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed/generated recombinantly. Recombinant polypeptides (e.g., xylanases) generated from these nucleic acids can be individually isolated or cloned and tested for a desired activity. Any recombinant expression system can be used, including bacterial, mammalian, yeast, insect or plant cell expression systems.

Alternatively, these nucleic acids can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68:109; Beaucage (1981) Tetra. Lett. 22:1859; U.S. Patent No. 4,458,066.

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Techniques for the manipulation of nucleic acids, such as, e.g., subcloning, labeling probes (e.g., random-primer labeling using Klenow polymerase, nick translation, amplification), sequencing, hybridization and the like are well described in the scientific and patent literature, see, e.g., Sambrook, ed., MOLECULAR CLONING: A LABORATORY MANUAL (2ND ED.), Vols. 1-3, Cold Spring Harbor Laboratory, (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, ed. John Wiley & Sons, Inc., New York (1997); LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY: HYBRIDIZATION WITH NUCLEIC ACID PROBES, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

Another useful means of obtaining and manipulating nucleic acids used to practice the methods of the invention is to clone from genomic samples, and, if desired, screen and re-clone inserts isolated or amplified from, e.g., genomic clones or cDNA clones. Sources of nucleic acid used in the methods of the invention include genomic or cDNA libraries contained in, e.g., mammalian artificial chromosomes (MACs), see, e.g., U.S. Patent Nos. 5,721,118; 6,025,155; human artificial chromosomes, see, e.g., Rosenfeld (1997) Nat. Genet. 15:333-335; yeast artificial chromosomes (YAC); bacterial artificial chromosomes (BAC); P1 artificial chromosomes, see, e.g., Woon (1998) Genomics 50:306-316; P1-derived vectors (PACs), see, e.g., Kern (1997) Biotechniques 23:120-124; cosmids, recombinant viruses, phages or plasmids.

In one aspect, a nucleic acid encoding a polypeptide of the invention is assembled in appropriate phase with a leader sequence capable of directing secretion of the translated polypeptide or fragment thereof.

The invention provides fusion proteins and nucleic acids encoding them. A polypeptide of the invention can be fused to a heterologous peptide or polypeptide, such as N-terminal identification peptides which impart desired characteristics, such as increased stability or simplified purification. Peptides and polypeptides of the invention can also be synthesized and expressed as fusion proteins with one or more additional domains linked thereto for, e.g., producing a more immunogenic peptide, to more readily isolate a

recombinantly synthesized peptide, to identify and isolate antibodies and antibody-expressing B cells, and the like. Detection and purification facilitating domains include, e.g., metal chelating peptides such as polyhistidine tracts and histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor Xa or enterokinase (Invitrogen, San Diego CA) between a purification domain and the motif-comprising peptide or polypeptide to facilitate purification. For example, an expression vector can include an epitope-encoding nucleic acid sequence linked to six histidine residues followed by a thioredoxin and an enterokinase cleavage site (see e.g., Williams (1995) Biochemistry 34:1787-1797; Dobeli (1998) Protein Expr. Purif. 12:404-414). The histidine residues facilitate detection and purification while the enterokinase cleavage site provides a means for purifying the epitope from the remainder of the fusion protein. Technology pertaining to vectors encoding fusion proteins and application of fusion proteins are well described in the scientific and patent literature, see e.g., Kroll (1993) DNA Cell. Biol., 12:441-53.

Transcriptional and translational control sequences

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The invention provides nucleic acid (e.g., DNA) sequences of the invention operatively linked to expression (e.g., transcriptional or translational) control sequence(s), e.g., promoters or enhancers, to direct or modulate RNA synthesis/ expression. The expression control sequence can be in an expression vector. Exemplary bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, PL and trp. Exemplary eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein I.

Promoters suitable for expressing a polypeptide in bacteria include the *E. coli* lac or trp promoters, the lacI promoter, the lacZ promoter, the T3 promoter, the T7 promoter, the gpt promoter, the lambda PR promoter, the lambda PL promoter, promoters from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), and the acid phosphatase promoter. Eukaryotic promoters include the CMV immediate early promoter, the HSV thymidine kinase promoter, heat shock promoters, the early and late SV40 promoter, LTRs from retroviruses, and the mouse metallothionein-I promoter. Other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses may also be used. Promoters suitable for expressing the polypeptide or fragment thereof in bacteria

include the $E.\ coli\ lac$ or trp promoters, the lacI promoter, the lacZ promoter, the T3 promoter, the T4 promoters from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK) and the acid phosphatase promoter. Fungal promoters include the t4 factor promoter. Eukaryotic promoters include the CMV immediate early promoter, the HSV thymidine kinase promoter, heat shock promoters, the early and late SV40 promoter, LTRs from retroviruses and the mouse metallothionein-I promoter. Other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses may also be used.

Tissue-Specific Plant Promoters

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The invention provides expression cassettes that can be expressed in a tissue-specific manner, e.g., that can express a xylanase of the invention in a tissue-specific manner. The invention also provides plants or seeds that express a xylanase of the invention in a tissue-specific manner. The tissue-specificity can be seed specific, stem specific, leaf specific, root specific, fruit specific and the like.

In one aspect, a constitutive promoter such as the CaMV 35S promoter can be used for expression in specific parts of the plant or seed or throughout the plant. For example, for overexpression, a plant promoter fragment can be employed which will direct expression of a nucleic acid in some or all tissues of a plant, e.g., a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'- promoter derived from T-DNA of Agrobacterium tumefaciens, and other transcription initiation regions from various plant genes known to those of skill. Such genes include, e.g., ACT11 from Arabidopsis (Huang (1996) Plant Mol. Biol. 33:125-139); Cat3 from Arabidopsis (GenBank No. U43147, Zhong (1996) Mol. Gen. Genet. 251:196-203); the gene encoding stearoyl-acyl carrier protein desaturase from Brassica napus (Genbank No. X74782, Solocombe (1994) Plant Physiol. 104:1167-1176); GPc1 from maize (GenBank No. X15596; Martinez (1989) J. Mol. Biol 208:551-565); the Gpc2 from maize (GenBank No. U45855, Manjunath (1997) Plant Mol. Biol. 33:97-112); plant promoters described in U.S. Patent Nos. 4,962,028; 5,633,440.

The invention uses tissue-specific or constitutive promoters derived from viruses which can include, e.g., the tobamovirus subgenomic promoter (Kumagai (1995)

Proc. Natl. Acad. Sci. USA 92:1679-1683; the rice tungro bacilliform virus (RTBV), which replicates only in phloem cells in infected rice plants, with its promoter which drives strong phloem-specific reporter gene expression; the cassava vein mosaic virus (CVMV) promoter, with highest activity in vascular elements, in leaf mesophyll cells, and in root tips (Verdaguer (1996) Plant Mol. Biol. 31:1129-1139).

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Alternatively, the plant promoter may direct expression of xylanase-expressing nucleic acid in a specific tissue, organ or cell type (*i.e.* tissue-specific promoters) or may be otherwise under more precise environmental or developmental control or under the control of an inducible promoter. Examples of environmental conditions that may affect transcription include anaerobic conditions, elevated temperature, the presence of light, or sprayed with chemicals/hormones. For example, the invention incorporates the drought-inducible promoter of maize (Busk (1997) supra); the cold, drought, and high salt inducible promoter from potato (Kirch (1997) Plant Mol. Biol. 33:897 909).

Tissue-specific promoters can promote transcription only within a certain time frame of developmental stage within that tissue. See, e.g., Blazquez (1998) Plant Cell 10:791-800, characterizing the Arabidopsis LEAFY gene promoter. See also Cardon (1997) Plant J 12:367-77, describing the transcription factor SPL3, which recognizes a conserved sequence motif in the promoter region of the A. thaliana floral meristem identity gene AP1; and Mandel (1995) Plant Molecular Biology, Vol. 29, pp 995-1004, describing the meristem promoter eIF4. Tissue specific promoters which are active throughout the life cycle of a particular tissue can be used. In one aspect, the nucleic acids of the invention are operably linked to a promoter active primarily only in cotton fiber cells. In one aspect, the nucleic acids of the invention are operably linked to a promoter active primarily during the stages of cotton fiber cell elongation, e.g., as described by Rinehart (1996) supra. The nucleic acids can be operably linked to the Fb12A gene promoter to be preferentially expressed in cotton fiber cells (Ibid). See also, John (1997) Proc. Natl. Acad. Sci. USA 89:5769-5773; John, et al., U.S. Patent Nos. 5,608,148 and 5,602,321, describing cotton fiber-specific promoters and methods for the construction of transgenic cotton plants. Root-specific promoters may also be used to express the nucleic acids of the invention. Examples of root-specific promoters include the promoter from the alcohol dehydrogenase gene (DeLisle (1990) Int. Rev. Cytol. 123:39-60). Other promoters that can be used to express the nucleic acids of the invention include, e.g., ovule-specific, embryo-specific, endosperm-specific, integument-specific, seed coat-specific promoters, or some combination thereof; a leaf-specific promoter (see, e.g., Busk (1997) Plant J. 11:1285 1295, describing a leaf-specific promoter in maize); the ORF13

promoter from Agrobacterium rhizogenes (which exhibits high activity in roots, see, e.g., Hansen (1997) supra); a maize pollen specific promoter (see, e.g., Guerrero (1990) Mol. Gen. Genet. 224:161 168); a tomato promoter active during fruit ripening, senescence and abscission of leaves and, to a lesser extent, of flowers can be used (see, e.g., Blume (1997) Plant J. 12:731 746); a pistil-specific promoter from the potato SK2 gene (see, e.g., Ficker (1997) Plant Mol. Biol. 35:425 431); the Blec4 gene from pea, which is active in epidermal tissue of vegetative and floral shoot apices of transgenic alfalfa making it a useful tool to target the expression of foreign genes to the epidermal layer of actively growing shoots or fibers; the ovule-specific BEL1 gene (see, e.g., Reiser (1995) Cell 83:735-742, GenBank No. U39944); and/or, the promoter in Klee, U.S. Patent No. 5,589,583, describing a plant promoter region is capable of conferring high levels of transcription in meristematic tissue and/or rapidly dividing cells.

Alternatively, plant promoters which are inducible upon exposure to plant hormones, such as auxins, are used to express the nucleic acids of the invention. For example, the invention can use the auxin-response elements E1 promoter fragment (AuxREs) in the soybean (Glycine max L.) (Liu (1997) Plant Physiol. 115:397-407); the auxin-responsive Arabidopsis GST6 promoter (also responsive to salicylic acid and hydrogen peroxide) (Chen (1996) Plant J. 10: 955-966); the auxin-inducible parC promoter from tobacco (Sakai (1996) 37:906-913); a plant biotin response element (Streit (1997) Mol. Plant Microbe Interact. 10:933-937); and, the promoter responsive to the stress hormone abscisic acid (Sheen (1996) Science 274:1900-1902).

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The nucleic acids of the invention can also be operably linked to plant promoters which are inducible upon exposure to chemicals reagents which can be applied to the plant, such as herbicides or antibiotics. For example, the maize In2-2 promoter, activated by benzenesulfonamide herbicide safeners, can be used (De Veylder (1997) Plant Cell Physiol. 38:568-577); application of different herbicide safeners induces distinct gene expression patterns, including expression in the root, hydathodes, and the shoot apical meristem. Coding sequence can be under the control of, e.g., a tetracycline-inducible promoter, e.g., as described with transgenic tobacco plants containing the Avena sativa L. (oat) arginine decarboxylase gene (Masgrau (1997) Plant J. 11:465-473); or, a salicylic acid-responsive element (Stange (1997) Plant J. 11:1315-1324). Using chemically- (e.g., hormone- or pesticide-) induced promoters, i.e., promoter responsive to a chemical which can be applied to the transgenic plant in the field, expression of a polypeptide of the invention can be induced at a particular stage of development of the plant. Thus, the invention also

provides for transgenic plants containing an inducible gene encoding for polypeptides of the invention whose host range is limited to target plant species, such as corn, rice, barley, wheat, potato or other crops, inducible at any stage of development of the crop.

One of skill will recognize that a tissue-specific plant promoter may drive expression of operably linked sequences in tissues other than the target tissue. Thus, a tissue-specific promoter is one that drives expression preferentially in the target tissue or cell type, but may also lead to some expression in other tissues as well.

The nucleic acids of the invention can also be operably linked to plant promoters which are inducible upon exposure to chemicals reagents. These reagents include, e.g., herbicides, synthetic auxins, or antibiotics which can be applied, e.g., sprayed, onto transgenic plants. Inducible expression of the xylanase-producing nucleic acids of the invention will allow the grower to select plants with the optimal xylanase expression and/or activity. The development of plant parts can thus controlled. In this way the invention provides the means to facilitate the harvesting of plants and plant parts. For example, in various embodiments, the maize In2-2 promoter, activated by benzenesulfonamide herbicide safeners, is used (De Veylder (1997) Plant Cell Physiol. 38:568-577); application of different herbicide safeners induces distinct gene expression patterns, including expression in the root, hydathodes, and the shoot apical meristem. Coding sequences of the invention are also under the control of a tetracycline-inducible promoter, e.g., as described with transgenic tobacco plants containing the *Avena sativa* L. (oat) arginine decarboxylase gene (Masgrau (1997) Plant J. 11:465-473); or, a salicylic acid-responsive element (Stange (1997) Plant J. 11:1315-1324).

In some aspects, proper polypeptide expression may require polyadenylation region at the 3'-end of the coding region. The polyadenylation region can be derived from the natural gene, from a variety of other plant (or animal or other) genes, or from genes in the Agrobacterial T-DNA.

Expression vectors and cloning vehicles

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The invention provides expression vectors and cloning vehicles comprising nucleic acids of the invention, e.g., sequences encoding the xylanases of the invention. Expression vectors and cloning vehicles of the invention can comprise viral particles, baculovirus, phage, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, viral DNA (e.g., vaccinia, adenovirus, foul pox virus, pseudorabies and derivatives of SV40), P1-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other

vectors specific for specific hosts of interest (such as bacillus, Aspergillus and yeast). Vectors of the invention can include chromosomal, non-chromosomal and synthetic DNA sequences. Large numbers of suitable vectors are known to those of skill in the art, and are commercially available. Exemplary vectors are include: bacterial: pQE vectors (Qiagen), pBluescript plasmids, pNH vectors, (lambda-ZAP vectors (Stratagene); ptrc99a, pKK223-3, pDR540, pRIT2T (Pharmacia); Eukaryotic: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, pSVLSV40 (Pharmacia). However, any other plasmid or other vector may be used so long as they are replicable and viable in the host. Low copy number or high copy number vectors may be employed with the present invention.

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The expression vector can comprise a promoter, a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. Mammalian expression vectors can comprise an origin of replication, any necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. In some aspects, DNA sequences derived from the SV40 splice and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

In one aspect, the expression vectors contain one or more selectable marker genes to permit selection of host cells containing the vector. Such selectable markers include genes encoding dihydrofolate reductase or genes conferring neomycin resistance for eukaryotic cell culture, genes conferring tetracycline or ampicillin resistance in *E. coli*, and the *S. cerevisiae* TRP1 gene. Promoter regions can be selected from any desired gene using chloramphenicol transferase (CAT) vectors or other vectors with selectable markers.

Vectors for expressing the polypeptide or fragment thereof in eukaryotic cells can also contain enhancers to increase expression levels. Enhancers are cis-acting elements of DNA, usually from about 10 to about 300 bp in length that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and the adenovirus enhancers.

A nucleic acid sequence can be inserted into a vector by a variety of procedures. In general, the sequence is ligated to the desired position in the vector following digestion of the insert and the vector with appropriate restriction endonucleases.

Alternatively, blunt ends in both the insert and the vector may be ligated. A variety of cloning techniques are known in the art, e.g., as described in Ausubel and Sambrook. Such procedures and others are deemed to be within the scope of those skilled in the art.

The vector can be in the form of a plasmid, a viral particle, or a phage. Other vectors include chromosomal, non-chromosomal and synthetic DNA sequences, derivatives of SV40; bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. A variety of cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by, e.g., Sambrook.

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Particular bacterial vectors which can be used include the commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017), pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden), GEM1 (Promega Biotec, Madison, WI, USA) pQE70, pQE60, pQE-9 (Qiagen), pD10, psiX174 pBluescript II KS, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene), ptrc99a, pKK223-3, pKK233-3, DR540, pRIT5 (Pharmacia), pKK232-8 and pCM7. Particular eukaryotic vectors include pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia). However, any other vector may be used as long as it is replicable and viable in the host cell.

The nucleic acids of the invention can be expressed in expression cassettes, vectors or viruses and transiently or stably expressed in plant cells and seeds. One exemplary transient expression system uses episomal expression systems, e.g., cauliflower mosaic virus (CaMV) viral RNA generated in the nucleus by transcription of an episomal minichromosome containing supercoiled DNA, see, e.g., Covey (1990) Proc. Natl. Acad. Sci. USA 87:1633-1637. Alternatively, coding sequences, i.e., all or sub-fragments of sequences of the invention can be inserted into a plant host cell genome becoming an integral part of the host chromosomal DNA. Sense or antisense transcripts can be expressed in this manner. A vector comprising the sequences (e.g., promoters or coding regions) from nucleic acids of the invention can comprise a marker gene that confers a selectable phenotype on a plant cell or a seed. For example, the marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to kanamycin, G418, bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosulfuron or Basta.

Expression vectors capable of expressing nucleic acids and proteins in plants are well known in the art, and can include, e.g., vectors from Agrobacterium spp., potato virus X (see, e.g., Angell (1997) EMBO J. 16:3675-3684), tobacco mosaic virus (see, e.g., Casper (1996) Gene 173:69-73), tomato bushy stunt virus (see, e.g., Hillman (1989) Virology 169:42-50), tobacco etch virus (see, e.g., Dolja (1997) Virology 234:243-252), bean golden mosaic virus (see, e.g., Morinaga (1993) Microbiol Immunol. 37:471-476), cauliflower mosaic virus (see, e.g., Cecchini (1997) Mol. Plant Microbe Interact. 10:1094-1101), maize

Ac/Ds transposable element (see, e.g., Rubin (1997) Mol. Cell. Biol. 17:6294-6302; Kunze (1996) Curr. Top. Microbiol. Immunol. 204:161-194), and the maize suppressor-mutator (Spm) transposable element (see, e.g., Schlappi (1996) Plant Mol. Biol. 32:717-725); and derivatives thereof.

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In one aspect, the expression vector can have two replication systems to allow it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a prokaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector can contain at least one sequence homologous to the host cell genome. It can contain two homologous sequences which flank the expression construct. The integrating vector can be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

Expression vectors of the invention may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed, e.g., genes which render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin and tetracycline. Selectable markers can also include biosynthetic genes, such as those in the histidine, tryptophan and leucine biosynthetic pathways.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct RNA synthesis. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression. Promoter regions can be selected from any desired gene using chloramphenical transferase (CAT) vectors or other vectors with selectable markers. In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

Mammalian expression vectors may also comprise an origin of replication, any necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences and 5' flanking nontranscribed sequences. In some

aspects, DNA sequences derived from the SV40 splice and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Vectors for expressing the polypeptide or fragment thereof in eukaryotic cells may also contain enhancers to increase expression levels. Enhancers are cis-acting elements of DNA, usually from about 10 to about 300 bp in length that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin and the adenovirus enhancers.

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In addition, the expression vectors typically contain one or more selectable marker genes to permit selection of host cells containing the vector. Such selectable markers include genes encoding dihydrofolate reductase or genes conferring neomycin resistance for eukaryotic cell culture, genes conferring tetracycline or ampicillin resistance in *E. coli* and the *S. cerevisiae TRP1* gene.

In some aspects, the nucleic acid encoding one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof is assembled in appropriate phase with a leader sequence capable of directing secretion of the translated polypeptide or fragment thereof. Optionally, the nucleic acid can encode a fusion polypeptide in which one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof is fused to heterologous peptides or polypeptides, such as N-terminal identification peptides which impart desired characteristics, such as increased stability or simplified purification.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is ligated to the desired position in the vector following digestion of the insert and the vector with appropriate restriction endonucleases. Alternatively, blunt ends in both the insert and the vector may be ligated. A variety of cloning techniques are disclosed in Ausubel et al. Current Protocols in Molecular Biology, John Wiley 503 Sons, Inc. 1997 and Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd Ed., Cold Spring Harbor Laboratory Press (1989. Such procedures and others are deemed to be within the scope of those skilled in the art.

The vector may be, for example, in the form of a plasmid, a viral particle, or a phage. Other vectors include chromosomal, nonchromosomal and synthetic DNA sequences, derivatives of SV40; bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors

derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus and pseudorabies. A variety of cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, N.Y., (1989).

Host cells and transformed cells

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The invention also provides a transformed cell comprising a nucleic acid sequence of the invention, e.g., a sequence encoding a xylanase of the invention, or a vector of the invention. The host cell may be any of the host cells familiar to those skilled in the art, including prokaryotic cells, eukaryotic cells, such as bacterial cells, fungal cells, yeast cells, mammalian cells, insect cells, or plant cells. Exemplary bacterial cells include *E. coli*, *Streptomyces*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*. Exemplary insect cells include *Drosophila S2* and *Spodoptera Sf9*. Exemplary animal cells include CHO, COS or Bowes melanoma or any mouse or human cell line. The selection of an appropriate host is within the abilities of those skilled in the art. Techniques for transforming a wide variety of higher plant species are well known and described in the technical and scientific literature. See, e.g., Weising (1988) Ann. Rev. Genet. 22:421-477; U.S. Patent No. 5,750,870.

The vector can be introduced into the host cells using any of a variety of techniques, including transformation, transfection, transduction, viral infection, gene guns, or Ti-mediated gene transfer. Particular methods include calcium phosphate transfection, DEAE-Dextran mediated transfection, lipofection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

In one aspect, the nucleic acids or vectors of the invention are introduced into the cells for screening, thus, the nucleic acids enter the cells in a manner suitable for subsequent expression of the nucleic acid. The method of introduction is largely dictated by the targeted cell type. Exemplary methods include CaPO₄ precipitation, liposome fusion, lipofection (e.g., LIPOFECTINTM), electroporation, viral infection, etc. The candidate nucleic acids may stably integrate into the genome of the host cell (for example, with retroviral introduction) or may exist either transiently or stably in the cytoplasm (i.e. through the use of traditional plasmids, utilizing standard regulatory sequences, selection markers, etc.). As many pharmaceutically important screens require human or model mammalian cell targets, retroviral vectors capable of transfecting such targets are can be used.

Where appropriate, the engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the invention. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter may be induced by appropriate means (e.g., temperature shift or chemical induction) and the cells may be cultured for an additional period to allow them to produce the desired polypeptide or fragment thereof.

Cells can be harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract is retained for further purification. Microbial cells employed for expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known to those skilled in the art. The expressed polypeptide or fragment thereof can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the polypeptide. If desired, high performance liquid chromatography (HPLC) can be employed for final purification steps.

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The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Depending upon the host employed in a recombinant production procedure, the polypeptides produced by host cells containing the vector may be glycosylated or may be non-glycosylated. Polypeptides of the invention may or may not also include an initial methionine amino acid residue.

Cell-free translation systems can also be employed to produce a polypeptide of the invention. Cell-free translation systems can use mRNAs transcribed from a DNA construct comprising a promoter operably linked to a nucleic acid encoding the polypeptide or fragment thereof. In some aspects, the DNA construct may be linearized prior to conducting an in vitro transcription reaction. The transcribed mRNA is then incubated with an appropriate cell-free translation extract, such as a rabbit reticulocyte extract, to produce the desired polypeptide or fragment thereof.

The expression vectors can contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate

reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

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Host cells containing the polynucleotides of interest, e.g., nucleic acids of the invention, can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression and will be apparent to the ordinarily skilled artisan. The clones which are identified as having the specified enzyme activity may then be sequenced to identify the polynucleotide sequence encoding an enzyme having the enhanced activity.

The invention provides a method for overexpressing a recombinant xylanase in a cell comprising expressing a vector comprising a nucleic acid of the invention, e.g., a nucleic acid comprising a nucleic acid sequence with at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to a sequence of Group A nucleic acid sequences over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection, or, a nucleic acid that hybridizes under stringent conditions to a nucleic acid sequence as set forth in Group A nucleic acid sequences, or a subsequence thereof. The overexpression can be effected by any means, e.g., use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.

The nucleic acids of the invention can be expressed, or overexpressed, in any in vitro or in vivo expression system. Any cell culture systems can be employed to express, or over-express, recombinant protein, including bacterial, insect, yeast, fungal or mammalian cultures. Over-expression can be effected by appropriate choice of promoters, enhancers, vectors (e.g., use of replicon vectors, dicistronic vectors (see, e.g., Gurtu (1996) Biochem. Biophys. Res. Commun. 229:295-8), media, culture systems and the like. In one aspect, gene amplification using selection markers, e.g., glutamine synthetase (see, e.g., Sanders (1987) Dev. Biol. Stand. 66:55-63), in cell systems are used to overexpress the polypeptides of the invention.

Additional details regarding this approach are in the public literature and/or are known to the skilled artisan. In a particular non-limiting exemplification, such publicly available literature includes EP 0659215 (W0 9403612 A1) (Nevalainen *et al.*); Lapidot, A., Mechaly, A., Shoham, Y., "Overexpression and single-step purification of a thermostable

xylanase from *Bacillus stearothermophilus* T-6," J. Biotechnol. Nov 51:259-64 (1996); Lüthi, E., Jasmat, N.B., Bergquist, P.L., "Xylanase from the extremely thermophilic bacterium *Caldocellum saccharolyticum*: overexpression of the gene in *Escherichia coli* and characterization of the gene product," Appl. Environ. Microbiol. Sep 56:2677-83 (1990); and Sung, W.L., Luk, C.K., Zahab, D.M., Wakarchuk, W., "Overexpression of the *Bacillus subtilis* and *circulans* xylanases in *Escherichia coli*," Protein Expr. Purif. Jun 4:200-6 (1993), although these references do not teach the inventive enzymes of the instant application.

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The host cell may be any of the host cells familiar to those skilled in the art, including prokaryotic cells, eukaryotic cells, mammalian cells, insect cells, or plant cells. As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as *E. coli*, *Streptomyces*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera Pseudomonas, Streptomyces and Staphylococcus, fungal cells, such as yeast, insect cells such as *Drosophila S2* and *Spodoptera Sf9*, animal cells such as CHO, COS or Bowes melanoma and adenoviruses. The selection of an appropriate host is within the abilities of those skilled in the art.

The vector may be introduced into the host cells using any of a variety of techniques, including transformation, transfection, transduction, viral infection, gene guns, or Ti-mediated gene transfer. Particular methods include calcium phosphate transfection, DEAE-Dextran mediated transfection, lipofection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

Where appropriate, the engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the invention. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter may be induced by appropriate means (e.g., temperature shift or chemical induction) and the cells may be cultured for an additional period to allow them to produce the desired polypeptide or fragment thereof.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means and the resulting crude extract is retained for further purification. Microbial cells employed for expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well known to those skilled in the art. The expressed polypeptide or fragment thereof can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation

exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the polypeptide. If desired, high performance liquid chromatography (HPLC) can be employed for final purification steps.

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Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts (described by Gluzman, Cell, 23:175, 1981) and other cell lines capable of expressing proteins from a compatible vector, such as the C127, 3T3, CHO, HeLa and BHK cell lines.

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Depending upon the host employed in a recombinant production procedure, the polypeptides produced by host cells containing the vector may be glycosylated or may be non-glycosylated. Polypeptides of the invention may or may not also include an initial methionine amino acid residue.

Alternatively, the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof can be synthetically produced by conventional peptide synthesizers. In other aspects, fragments or portions of the polypeptides may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides.

Cell-free translation systems can also be employed to produce one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof using mRNAs transcribed from a DNA construct comprising a promoter operably linked to a nucleic acid encoding the polypeptide or fragment thereof. In some aspects, the DNA construct may be linearized prior to conducting an *in vitro* transcription reaction. The transcribed mRNA is then incubated with an appropriate cell-free translation extract, such as a rabbit reticulocyte extract, to produce the desired polypeptide or fragment thereof.

Amplification of Nucleic Acids

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In practicing the invention, nucleic acids of the invention and nucleic acids encoding the xylanases of the invention, or modified nucleic acids of the invention, can be reproduced by amplification. Amplification can also be used to clone or modify the nucleic acids of the invention. Thus, the invention provides amplification primer sequence pairs for amplifying nucleic acids of the invention. One of skill in the art can design amplification primer sequence pairs for any part of or the full length of these sequences.

In one aspect, the invention provides a nucleic acid amplified by a primer pair of the invention, e.g., a primer pair as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of a nucleic acid of the invention, and about the first (the 5') 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of the complementary strand.

The invention provides an amplification primer sequence pair for amplifying a nucleic acid encoding a polypeptide having a xylanase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence of the invention, or fragments or subsequences thereof. One or each member of the amplification primer sequence pair can comprise an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence, or about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 consecutive bases of the sequence. The invention provides amplification primer pairs, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of a nucleic acid of the invention, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 residues of the complementary strand of the first member. The invention provides xylanases generated by amplification, e.g., polymerase chain reaction (PCR), using an amplification primer pair of the invention. The invention provides methods of making a xylanase by amplification, e.g., polymerase chain reaction (PCR), using an amplification primer pair of the invention. In one aspect, the amplification primer pair amplifies a nucleic acid from a library, e.g., a gene library, such as an environmental library.

Amplification reactions can also be used to quantify the amount of nucleic acid in a sample (such as the amount of message in a cell sample), label the nucleic acid (e.g., to apply it to an array or a blot), detect the nucleic acid, or quantify the amount of a specific nucleic acid in a sample. In one aspect of the invention, message isolated from a cell or a cDNA library are amplified.

The skilled artisan can select and design suitable oligonucleotide amplification primers. Amplification methods are also well known in the art, and include, e.g., polymerase chain reaction, PCR (see, e.g., PCR PROTOCOLS, A GUIDE TO METHODS AND APPLICATIONS, ed. Innis, Academic Press, N.Y. (1990) and PCR STRATEGIES (1995), 5 ed. Innis, Academic Press, Inc., N.Y., ligase chain reaction (LCR) (see, e.g., Wu (1989) Genomics 4:560; Landegren (1988) Science 241:1077; Barringer (1990) Gene 89:117); transcription amplification (see, e.g., Kwoh (1989) Proc. Natl. Acad. Sci. USA 86:1173); and, self-sustained sequence replication (see, e.g., Guatelli (1990) Proc. Natl. Acad. Sci. USA 87:1874); Q Beta replicase amplification (see, e.g., Smith (1997) J. Clin. Microbiol. 35:1477-10 1491), automated Q-beta replicase amplification assay (see, e.g., Burg (1996) Mol. Cell. Probes 10:257-271) and other RNA polymerase mediated techniques (e.g., NASBA, Cangene, Mississauga, Ontario); see also Berger (1987) Methods Enzymol. 152:307-316; Sambrook; Ausubel; U.S. Patent Nos. 4,683,195 and 4,683,202; Sooknanan (1995) Biotechnology 13:563-564.

15 Determining the degree of sequence identity

The invention provides nucleic acids comprising sequences having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 20 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary nucleic acid of the invention (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEO ID NO:25, SEO ID NO:27, SEO ID NO:29, SEO ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEO ID NO:41, 25 SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEO ID NO:73, SEO ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID 30 NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ.ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID

NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEO ID NO:149, SEO ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID 5 NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEO ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEO ID NO:229, SEO ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID 10 NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEO ID NO:259, SEO ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID 15 NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID 20 NO:327, SEO ID NO:329, SEO ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEO ID NO:359, SEO ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379) over a region of at least about 50, 75, 100, 150, 200, 250, 300, 25 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550 or more, residues. The invention provides polypeptides comprising sequences having at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 30 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary polypeptide of the invention. The extent of sequence identity (homology) may be determined using any computer program and

associated parameters, including those described herein, such as BLAST 2.2.2. or FASTA version 3.0t78, with the default parameters.

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The nucleic acid sequences are also referred to as "Group A" nucleic acid sequences, which include sequences substantially identical thereto, as well as sequences homologous to Group A nucleic acid sequences and fragments thereof and sequences complementary to all of the preceding sequences. Nucleic acid sequences of the invention can comprise at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of an exemplary sequence of the invention (e.g., Group A nucleic acid sequences) and sequences substantially identical thereto. Homologous sequences and fragments of Group A nucleic acid sequences and sequences substantially identical thereto, refer to a sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, or 50% homology to these sequences. Homology may be determined using any of the computer programs and parameters described herein, including FASTA version 3.0t78 with the default parameters. Homologous sequences also include RNA sequences in which uridines replace the thymines in the nucleic acid sequences as set forth in the Group A nucleic acid sequences. The homologous sequences may be obtained using any of the procedures described herein or may result from the correction of a sequencing error. It will be appreciated that the nucleic acid sequences as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, can be represented in the traditional single character format (See the inside back cover of Stryer, Lubert. Biochemistry, 3rd Ed., W. H. Freeman & Co., New York.) or in any other format which records the identity of the nucleotides in a sequence.

Various sequence comparison programs identified elsewhere in this patent specification are particularly contemplated for use in this aspect of the invention. Protein and/or nucleic acid sequence homologies may be evaluated using any of the variety of sequence comparison algorithms and programs known in the art. Such algorithms and programs include, but are by no means limited to, TBLASTN, BLASTP, FASTA, TFASTA and CLUSTALW (Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85(8):2444-2448, 1988; Altschul et al., J. Mol. Biol. 215(3):403-410, 1990; Thompson et al., Nucleic Acids Res. 22(2):4673-4680, 1994; Higgins et al., Methods Enzymol. 266:383-402, 1996; Altschul et al., J. Mol. Biol. 215(3):403-410, 1990; Altschul et al., Nature Genetics 3:266-272, 1993).

Homology or identity is often measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches

similar sequences by assigning degrees of homology to various deletions, substitutions and other modifications. The terms "homology" and "identity" in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same when compared and aligned for maximum correspondence over a comparison window or designated region as measured using any number of sequence comparison algorithms or by manual alignment and visual inspection.

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For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequence for comparison are wellknown in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482, 1981, by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol 48:443, 1970, by the search for similarity method of person & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444, 1988, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection. Other algorithms for determining homology or identity include, for example, in addition to a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information), ALIGN, AMAS (Analysis of Multiply Aligned Sequences), AMPS (Protein Multiple Sequence Alignment), ASSET (Aligned Segment Statistical Evaluation Tool), BANDS, BESTSCOR, BIOSCAN (Biological Sequence Comparative Analysis Node), BLIMPS (BLocks IMProved Searcher), FASTA, Intervals & Points, BMB, CLUSTAL V, CLUSTAL W, CONSENSUS, LCONSENSUS, WCONSENSUS, Smith-Waterman algorithm, DARWIN, Las Vegas algorithm, FNAT (Forced Nucleotide Alignment Tool), Framealign, Framesearch,

DYNAMIC, FILTER, FSAP (Fristensky Sequence Analysis Package), GAP (Global Alignment Program), GENAL, GIBBS, GenQuest, ISSC (Sensitive Sequence Comparison), LALIGN (Local Sequence Alignment), LCP (Local Content Program), MACAW (Multiple Alignment Construction & Analysis Workbench), MAP (Multiple Alignment Program),

- MBLKP, MBLKN, PIMA (Pattern-Induced Multi-sequence Alignment), SAGA (Sequence Alignment by Genetic Algorithm) and WHAT-IF. Such alignment programs can also be used to screen genome databases to identify polynucleotide sequences having substantially identical sequences. A number of genome databases are available, for example, a substantial portion of the human genome is available as part of the Human Genome Sequencing Project (J.
- 10 Roach, http://weber.u.Washington.edu/~roach/human_genome_progress 2.html) (Gibbs, 1995). At least twenty-one other genomes have already been sequenced, including, for example, M. genitalium (Fraser et al., 1995), M. jannaschii (Bult et al., 1996), H. influenzae (Fleischmann et al., 1995), E. coli (Blattner et al., 1997) and yeast (S. cerevisiae) (Mewes et al., 1997) and D. melanogaster (Adams et al., 2000). Significant progress has also been made in sequencing the genomes of model organism, such as mouse, C. elegans and Arabadopsis sp. Several databases containing genomic information annotated with some functional information are maintained by different organization and are accessible via the internet

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One example of a useful algorithm is BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977 and Altschul et al., J. Mol. Biol. 215:403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X

determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3 and expectations (E) of 10 and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915, 1989) alignments (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

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The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Natl. Acad. Sci. USA 90:5873, 1993). One measure of similarity provided by BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a references sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01 and most preferably less than about 0.001.

In one aspect, protein and nucleic acid sequence homologies are evaluated using the Basic Local Alignment Search Tool ("BLAST") In particular, five specific BLAST programs are used to perform the following task:

- (1) BLASTP and BLAST3 compare an amino acid query sequence against a protein sequence database;
- (2) BLASTN compares a nucleotide query sequence against a nucleotide sequence database;
- (3) BLASTX compares the six-frame conceptual translation products of a query nucleotide sequence (both strands) against a protein sequence database;
- (4) TBLASTN compares a query protein sequence against a nucleotide sequence database translated in all six reading frames (both strands); and
- (5) TBLASTX compares the six-frame translations of a nucleotide query sequence against the six-frame translations of a nucleotide sequence database.

The BLAST programs identify homologous sequences by identifying similar segments, which are referred to herein as "high-scoring segment pairs," between a query amino or nucleic acid sequence and a test sequence which is preferably obtained from a protein or nucleic acid sequence database. High-scoring segment pairs are preferably identified (i.e., aligned) by means of a scoring matrix, many of which are known in the art. Preferably, the scoring matrix used is the BLOSUM62 matrix (Gonnet et al., Science 256:1443-1445, 1992; Henikoff and Henikoff, Proteins 17:49-61, 1993). Less preferably, the

PAM or PAM250 matrices may also be used (see, e.g., Schwartz and Dayhoff, eds., 1978, Matrices for Detecting Distance Relationships: Atlas of Protein Sequence and Structure, Washington: National Biomedical Research Foundation). BLAST programs are accessible through the U.S. National Library of Medicine.

The parameters used with the above algorithms may be adapted depending on the sequence length and degree of homology studied. In some aspects, the parameters may be the default parameters used by the algorithms in the absence of instructions from the user.

Computer systems and computer program products

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To determine and identify sequence identities, structural homologies, motifs and the like in silico, a nucleic acid or polypeptide sequence of the invention can be stored, recorded, and manipulated on any medium which can be read and accessed by a computer.

Accordingly, the invention provides computers, computer systems, computer readable mediums, computer programs products and the like recorded or stored thereon the nucleic acid and polypeptide sequences of the invention. As used herein, the words "recorded" and "stored" refer to a process for storing information on a computer medium. A skilled artisan can readily adopt any known methods for recording information on a computer readable medium to generate manufactures comprising one or more of the nucleic acid and/or polypeptide sequences of the invention.

B amino acid sequences, the exemplary sequences of the invention, and sequences substantially identical thereto, and fragments of any of the preceding sequences.

Substantially identical, or homologous, polypeptide sequences refer to a polypeptide sequence having at least 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity to an exemplary sequence of the invention, e.g., a polypeptide sequences of the Group B amino acid sequences.

Homology may be determined using any of the computer programs and parameters described herein, including FASTA version 3.0t78 with the default parameters or with any modified parameters. The homologous sequences may be obtained using any of the procedures described herein or may result from the correction of a sequencing error. The polypeptide fragments comprise at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150,

200, 250, 300, 350, 400, 450, 500 or more consecutive amino acids of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto. It will be appreciated that the polypeptide codes as set forth in Group B amino acid sequences and sequences substantially identical thereto, can be represented in the traditional single character format or three letter format (See the inside back cover of Stryer, Lubert. <u>Biochemistry</u>, 3rd Ed., W. H Freeman & Co., New York.) or in any other format which relates the identity of the polypeptides in a sequence.

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A nucleic acid or polypeptide sequence of the invention can be stored, recorded and manipulated on any medium which can be read and accessed by a computer. As used herein, the words "recorded" and "stored" refer to a process for storing information on a computer medium. A skilled artisan can readily adopt any of the presently known methods for recording information on a computer readable medium to generate manufactures comprising one or more of the nucleic acid sequences as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, one or more of the polypeptide sequences as set forth in Group B amino acid sequences and sequences substantially identical thereto. Another aspect of the invention is a computer readable medium having recorded thereon at least 2, 5, 10, 15, or 20 or more nucleic acid sequences as set forth in Group A nucleic acid sequences and sequences substantially identical thereto.

Another aspect of the invention is a computer readable medium having recorded thereon one or more of the nucleic acid sequences as set forth in Group A nucleic acid sequences and sequences substantially identical thereto. Another aspect of the invention is a computer readable medium having recorded thereon one or more of the polypeptide sequences as set forth in Group B amino acid sequences and sequences substantially identical thereto. Another aspect of the invention is a computer readable medium having recorded thereon at least 2, 5, 10, 15, or 20 or more of the sequences as set forth above.

Computer readable media include magnetically readable media, optically readable media, electronically readable media and magnetic/optical media. For example, the computer readable media may be a hard disk, a floppy disk, a magnetic tape, CD-ROM, Digital Versatile Disk (DVD), Random Access Memory (RAM), or Read Only Memory (ROM) as well as other types of other media known to those skilled in the art.

Aspects of the invention include systems (e.g., internet based systems), particularly computer systems which store and manipulate the sequence information described herein. One example of a computer system 100 is illustrated in block diagram form in Figure 1. As used herein, "a computer system" refers to the hardware components, software components

and data storage components used to analyze a nucleotide sequence of a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in the Group B amino acid sequences. The computer system 100 typically includes a processor for processing, accessing and manipulating the sequence data. The processor 105 can be any well-known type of central processing unit, such as, for example, the Pentium III from Intel Corporation, or similar processor from Sun, Motorola, Compaq, AMD or International Business Machines.

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Typically the computer system 100 is a general purpose system that comprises the processor 105 and one or more internal data storage components 110 for storing data and one or more data retrieving devices for retrieving the data stored on the data storage components. A skilled artisan can readily appreciate that any one of the currently available computer systems are suitable.

In one particular aspect, the computer system 100 includes a processor 105 connected to a bus which is connected to a main memory 115 (preferably implemented as RAM) and one or more internal data storage devices 110, such as a hard drive and/or other computer readable media having data recorded thereon. In some aspects, the computer system 100 further includes one or more data retrieving device 118 for reading the data stored on the internal data storage devices 110.

The data retrieving device 118 may represent, for example, a floppy disk drive, a compact disk drive, a magnetic tape drive, or a modem capable of connection to a remote data storage system (e.g., via the internet) etc. In some aspects, the internal data storage device 110 is a removable computer readable medium such as a floppy disk, a compact disk, a magnetic tape, etc. containing control logic and/or data recorded thereon. The computer system 100 may advantageously include or be programmed by appropriate software for reading the control logic and/or the data from the data storage component once inserted in the data retrieving device.

The computer system 100 includes a display 120 which is used to display output to a computer user. It should also be noted that the computer system 100 can be linked to other computer systems 125a-c in a network or wide area network to provide centralized access to the computer system 100.

Software for accessing and processing the nucleotide sequences of a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, (such as search tools, compare tools and modeling tools etc.) may reside in main memory 115 during execution.

In some aspects, the computer system 100 may further comprise a sequence comparison algorithm for comparing a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, stored on a computer readable medium to a reference nucleotide or polypeptide sequence(s) stored on a computer readable medium. A "sequence comparison algorithm" refers to one or more programs which are implemented (locally or remotely) on the computer system 100 to compare a nucleotide sequence with other nucleotide sequences and/or compounds stored within a data storage means. For example, the sequence comparison algorithm may compare the nucleotide sequences of a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, stored on a computer readable medium to reference sequences stored on a computer readable medium to identify homologies or structural motifs.

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Figure 2 is a flow diagram illustrating one aspect of a process 200 for comparing a new nucleotide or protein sequence with a database of sequences in order to determine the homology levels between the new sequence and the sequences in the database. The database of sequences can be a private database stored within the computer system 100, or a public database such as GENBANK that is available through the Internet.

The process 200 begins at a start state 201 and then moves to a state 202 wherein the new sequence to be compared is stored to a memory in a computer system 100. As discussed above, the memory could be any type of memory, including RAM or an internal storage device.

The process 200 then moves to a state 204 wherein a database of sequences is opened for analysis and comparison. The process 200 then moves to a state 206 wherein the first sequence stored in the database is read into a memory on the computer. A comparison is then performed at a state 210 to determine if the first sequence is the same as the second sequence. It is important to note that this step is not limited to performing an exact comparison between the new sequence and the first sequence in the database. Well-known methods are known to those of skill in the art for comparing two nucleotide or protein sequences, even if they are not identical. For example, gaps can be introduced into one sequence in order to raise the homology level between the two tested sequences. The parameters that control whether gaps or other features are introduced into a sequence during comparison are normally entered by the user of the computer system.

Once a comparison of the two sequences has been performed at the state 210, a determination is made at a decision state 210 whether the two sequences are the same. Of course, the term "same" is not limited to sequences that are absolutely identical. Sequences that are within the homology parameters entered by the user will be marked as "same" in the process 200.

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If a determination is made that the two sequences are the same, the process 200 moves to a state 214 wherein the name of the sequence from the database is displayed to the user. This state notifies the user that the sequence with the displayed name fulfills the homology constraints that were entered. Once the name of the stored sequence is displayed to the user, the process 200 moves to a decision state 218 wherein a determination is made whether more sequences exist in the database. If no more sequences exist in the database, then the process 200 terminates at an end state 220. However, if more sequences do exist in the database, then the process 200 moves to a state 224 wherein a pointer is moved to the next sequence in the database so that it can be compared to the new sequence. In this manner, the new sequence is aligned and compared with every sequence in the database.

It should be noted that if a determination had been made at the decision state 212 that the sequences were not homologous, then the process 200 would move immediately to the decision state 218 in order to determine if any other sequences were available in the database for comparison.

Accordingly, one aspect of the invention is a computer system comprising a processor, a data storage device having stored thereon a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, a data storage device having retrievably stored thereon reference nucleotide sequences or polypeptide sequences to be compared to a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto and a sequence comparer for conducting the comparison. The sequence comparer may indicate a homology level between the sequences compared or identify structural motifs in the above described nucleic acid code of Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, or it may identify structural motifs in sequences which are compared to these nucleic acid codes and polypeptide codes. In some aspects, the data storage device may have stored thereon the

sequences of at least 2, 5, 10, 15, 20, 25, 30 or 40 or more of the nucleic acid sequences as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or the polypeptide sequences as set forth in Group B amino acid sequences and sequences substantially identical thereto.

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Another aspect of the invention is a method for determining the level of homology between a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto and a reference nucleotide sequence. The method including reading the nucleic acid code or the polypeptide code and the reference nucleotide or polypeptide sequence through the use of a computer program which determines homology levels and determining homology between the nucleic acid code or polypeptide code and the reference nucleotide or polypeptide sequence with the computer program. The computer program may be any of a number of computer programs for determining homology levels, including those specifically enumerated herein, (e.g., BLAST2N with the default parameters or with any modified parameters). The method may be implemented using the computer systems described above. The method may also be performed by reading at least 2, 5, 10, 15, 20, 25, 30 or 40 or more of the above described nucleic acid sequences as set forth in the Group A nucleic acid sequences, or the polypeptide sequences as set forth in the Group B amino acid sequences through use of the computer program and determining homology between the nucleic acid codes or polypeptide codes and reference nucleotide sequences or polypeptide sequences.

Figure 3 is a flow diagram illustrating one aspect of a process 250 in a computer for determining whether two sequences are homologous. The process 250 begins at a start state 252 and then moves to a state 254 wherein a first sequence to be compared is stored to a memory. The second sequence to be compared is then stored to a memory at a state 256. The process 250 then moves to a state 260 wherein the first character in the first sequence is read and then to a state 262 wherein the first character of the second sequence is read. It should be understood that if the sequence is a nucleotide sequence, then the character would normally be either A, T, C, G or U. If the sequence is a protein sequence, then it is preferably in the single letter amino acid code so that the first and sequence sequences can be easily compared.

A determination is then made at a decision state 264 whether the two characters are the same. If they are the same, then the process 250 moves to a state 268 wherein the next characters in the first and second sequences are read. A determination is

then made whether the next characters are the same. If they are, then the process 250 continues this loop until two characters are not the same. If a determination is made that the next two characters are not the same, the process 250 moves to a decision state 274 to determine whether there are any more characters either sequence to read.

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If there are not any more characters to read, then the process 250 moves to a state 276 wherein the level of homology between the first and second sequences is displayed to the user. The level of homology is determined by calculating the proportion of characters between the sequences that were the same out of the total number of sequences in the first sequence. Thus, if every character in a first 100 nucleotide sequence aligned with a every character in a second sequence, the homology level would be 100%.

Alternatively, the computer program may be a computer program which compares the nucleotide sequences of a nucleic acid sequence as set forth in the invention, to one or more reference nucleotide sequences in order to determine whether the nucleic acid code of Group A nucleic acid sequences and sequences substantially identical thereto, differs from a reference nucleic acid sequence at one or more positions. Optionally such a program records the length and identity of inserted, deleted or substituted nucleotides with respect to the sequence of either the reference polynucleotide or a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto. In one aspect, the computer program may be a program which determines whether a nucleic acid sequence as set forth in Group A nucleic acid sequences substantially identical thereto, contains a single nucleotide polymorphism (SNP) with respect to a reference nucleotide sequence.

Accordingly, another aspect of the invention is a method for determining whether a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, differs at one or more nucleotides from a reference nucleotide sequence comprising the steps of reading the nucleic acid code and the reference nucleotide sequence through use of a computer program which identifies differences between nucleic acid sequences and identifying differences between the nucleic acid code and the reference nucleotide sequence with the computer program. In some aspects, the computer program is a program which identifies single nucleotide polymorphisms. The method may be implemented by the computer systems described above and the method illustrated in Figure 3. The method may also be performed by reading at least 2, 5, 10, 15, 20, 25, 30, or 40 or more of the nucleic acid sequences as set forth in Group A nucleic acid sequences and sequences substantially identical thereto and the reference nucleotide sequences through the use of the

computer program and identifying differences between the nucleic acid codes and the reference nucleotide sequences with the computer program.

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In other aspects the computer based system may further comprise an identifier for identifying features within a nucleic acid sequence as set forth in the Group A nucleic acid sequences or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto.

An "identifier" refers to one or more programs which identifies certain features within a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto. In one aspect, the identifier may comprise a program which identifies an open reading frame in a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto.

Figure 4 is a flow diagram illustrating one aspect of an identifier process 300 for detecting the presence of a feature in a sequence. The process 300 begins at a start state 302 and then moves to a state 304 wherein a first sequence that is to be checked for features is stored to a memory 115 in the computer system 100. The process 300 then moves to a state 306 wherein a database of sequence features is opened. Such a database would include a list of each feature's attributes along with the name of the feature. For example, a feature name could be "Initiation Codon" and the attribute would be "ATG". Another example would be the feature name "TAATAA Box" and the feature attribute would be "TAATAA". An example of such a database is produced by the University of Wisconsin Genetics Computer Group. Alternatively, the features may be structural polypeptide motifs such as alpha helices, beta sheets, or functional polypeptide motifs such as enzymatic active sites, helix-turn-helix motifs or other motifs known to those skilled in the art.

Once the database of features is opened at the state 306, the process 300 moves to a state 308 wherein the first feature is read from the database. A comparison of the attribute of the first feature with the first sequence is then made at a state 310. A determination is then made at a decision state 316 whether the attribute of the feature was found in the first sequence. If the attribute was found, then the process 300 moves to a state 318 wherein the name of the found feature is displayed to the user.

The process 300 then moves to a decision state 320 wherein a determination is made whether move features exist in the database. If no more features do exist, then the process 300 terminates at an end state 324. However, if more features do exist in the

database, then the process 300 reads the next sequence feature at a state 326 and loops back to the state 310 wherein the attribute of the next feature is compared against the first sequence. It should be noted, that if the feature attribute is not found in the first sequence at the decision state 316, the process 300 moves directly to the decision state 320 in order to determine if any more features exist in the database.

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Accordingly, another aspect of the invention is a method of identifying a feature within a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, comprising reading the nucleic acid code(s) or polypeptide code(s) through the use of a computer program which identifies features therein and identifying features within the nucleic acid code(s) with the computer program. In one aspect, computer program comprises a computer program which identifies open reading frames. The method may be performed by reading a single sequence or at least 2, 5, 10, 15, 20, 25, 30, or 40 of the nucleic acid sequences as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or the polypeptide sequences as set forth in Group B amino acid sequences and sequences substantially identical thereto, through the use of the computer program and identifying features within the nucleic acid codes or polypeptide codes with the computer program.

A nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, may be stored and manipulated in a variety of data processor programs in a variety of formats. For example, a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto, may be stored as text in a word processing file, such as Microsoft WORDTM or WORDPERFECTTM or as an ASCII file in a variety of database programs familiar to those of skill in the art, such as DB2TM, SYBASETM, or ORACLETM. In addition, many computer programs and databases may be used as sequence comparison algorithms, identifiers, or sources of reference nucleotide sequences or polypeptide sequences to be compared to a nucleic acid sequence as set forth in Group A nucleic acid sequences and sequences substantially identical thereto, or a polypeptide sequence as set forth in Group B amino acid sequences and sequences substantially identical thereto. The following list is intended not to limit the invention but to provide guidance to programs and databases which are useful with the nucleic acid sequences as set forth in Group A nucleic acid

sequences and sequences substantially identical thereto, or the polypeptide sequences as set forth in Group B amino acid sequences and sequences substantially identical thereto.

The programs and databases which may be used include, but are not limited to: MacPattern (EMBL), DiscoveryBase (Molecular Applications Group), GeneMine (Molecular Applications Group), Look (Molecular Applications Group), MacLook (Molecular Applications 5 Group), BLAST and BLAST2 (NCBI), BLASTN and BLASTX (Altschul et al, J. Mol. Biol. 215: 403, 1990), FASTA (Pearson and Lipman, Proc. Natl. Acad. Sci. USA, 85: 2444, 1988), FASTDB (Brutlag et al. Comp. App. Biosci. 6:237-245, 1990), Catalyst (Molecular Simulations Inc.), Catalyst/SHAPE (Molecular Simulations Inc.), Cerius².DBAccess (Molecular Simulations 10 Inc.), HypoGen (Molecular Simulations Inc.), Insight II, (Molecular Simulations Inc.), Discover (Molecular Simulations Inc.), CHARMm (Molecular Simulations Inc.), Felix (Molecular Simulations Inc.), DelPhi, (Molecular Simulations Inc.), QuanteMM, (Molecular Simulations Inc.), Homology (Molecular Simulations Inc.), Modeler (Molecular Simulations Inc.), ISIS (Molecular Simulations Inc.), Quanta/Protein Design (Molecular Simulations Inc.), WebLab 15 (Molecular Simulations Inc.), WebLab Diversity Explorer (Molecular Simulations Inc.), Gene Explorer (Molecular Simulations Inc.), SeqFold (Molecular Simulations Inc.), the MDL Available Chemicals Directory database, the MDL Drug Data Report data base, the Comprehensive Medicinal Chemistry database, Derwents's World Drug Index database, the BioByteMasterFile database, the Genbank database and the Genseqn database. Many other 20 programs and data bases would be apparent to one of skill in the art given the present disclosure.

Motifs which may be detected using the above programs include sequences encoding leucine zippers, helix-turn-helix motifs, glycosylation sites, ubiquitination sites, alpha helices and beta sheets, signal sequences encoding signal peptides which direct the secretion of the encoded proteins, sequences implicated in transcription regulation such as homeoboxes, acidic stretches, enzymatic active sites, substrate binding sites and enzymatic cleavage sites.

Hybridization of nucleic acids

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The invention provides isolated or recombinant nucleic acids that hybridize under stringent conditions to an exemplary sequence of the invention (e.g., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45,

SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, 5 SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID 10 NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID 15 NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID 20 NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID 25 NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID 30 NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379). The stringent conditions can be highly stringent conditions, medium stringent conditions and/or

low stringent conditions, including the high and reduced stringency conditions described herein. In one aspect, it is the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is within the scope of the invention, as discussed below.

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In alternative aspects, nucleic acids of the invention as defined by their ability to hybridize under stringent conditions can be between about five residues and the full length of nucleic acid of the invention; e.g., they can be at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 55, 60, 65, 70, 75, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, or more, residues in length. Nucleic acids shorter than full length are also included. These nucleic acids can be useful as, e.g., hybridization probes, labeling probes, PCR oligonucleotide probes, iRNA (single or double stranded), antisense or sequences encoding antibody binding peptides (epitopes), motifs, active sites and the like.

In one aspect, nucleic acids of the invention are defined by their ability to hybridize under high stringency comprises conditions of about 50% formamide at about 37°C to 42°C. In one aspect, nucleic acids of the invention are defined by their ability to hybridize under reduced stringency comprising conditions in about 35% to 25% formamide at about 30°C to 35°C.

Alternatively, nucleic acids of the invention are defined by their ability to hybridize under high stringency comprising conditions at 42°C in 50% formamide, 5X SSPE, 0.3% SDS, and a repetitive sequence blocking nucleic acid, such as cot-1 or salmon sperm DNA (e.g., 200 n/ml sheared and denatured salmon sperm DNA). In one aspect, nucleic acids of the invention are defined by their ability to hybridize under reduced stringency conditions comprising 35% formamide at a reduced temperature of 35°C.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (e.g., GC v. AT content) and nucleic acid type (e.g., RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

Hybridization may be carried out under conditions of low stringency, moderate stringency or high stringency. As an example of nucleic acid hybridization, a polymer membrane containing immobilized denatured nucleic acids is first prehybridized for 30

minutes at 45°C in a solution consisting of 0.9 M NaCl, 50 mM NaH₂PO₄, pH 7.0, 5.0 mM Na₂EDTA, 0.5% SDS, 10X Denhardt's and 0.5 mg/ml polyriboadenylic acid. Approximately 2 X 10⁷ cpm (specific activity 4-9 X 10⁸ cpm/ug) of ³²P end-labeled oligonucleotide probe are then added to the solution. After 12-16 hours of incubation, the membrane is washed for 30 minutes at room temperature in 1X SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA) containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at T_m-10°C for the oligonucleotide probe. The membrane is then exposed to autoradiographic film for detection of hybridization signals.

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All of the foregoing hybridizations would be considered to be under conditions of high stringency.

Following hybridization, a filter can be washed to remove any non-specifically bound detectable probe. The stringency used to wash the filters can also be varied depending on the nature of the nucleic acids being hybridized, the length of the nucleic acids being hybridized, the degree of complementarity, the nucleotide sequence composition (e.g., GC v. AT content) and the nucleic acid type (e.g., RNA v. DNA). Examples of progressively higher stringency condition washes are as follows: 2X SSC, 0.1% SDS at room temperature for 15 minutes (low stringency); 0.1X SSC, 0.5% SDS at room temperature for 30 minutes to 1 hour (moderate stringency); 0.1X SSC, 0.5% SDS for 15 to 30 minutes at between the hybridization temperature and 68°C (high stringency); and 0.15M NaCl for 15 minutes at 72°C (very high stringency). A final low stringency wash can be conducted in 0.1X SSC at room temperature. The examples above are merely illustrative of one set of conditions that can be used to wash filters. One of skill in the art would know that there are numerous recipes for different stringency washes. Some other examples are given below.

Nucleic acids which have hybridized to the probe are identified by autoradiography or other conventional techniques.

The above procedure may be modified to identify nucleic acids having decreasing levels of homology to the probe sequence. For example, to obtain nucleic acids of decreasing homology to the detectable probe, less stringent conditions may be used. For example, the hybridization temperature may be decreased in increments of 5°C from 68°C to 42°C in a hybridization buffer having a Na+ concentration of approximately 1M. Following hybridization, the filter may be washed with 2X SSC, 0.5% SDS at the temperature of hybridization. These conditions are considered to be "moderate" conditions above 50°C and "low" conditions below 50°C. A specific example of "moderate" hybridization conditions is

when the above hybridization is conducted at 55°C. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 45°C.

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Alternatively, the hybridization may be carried out in buffers, such as 6X SSC, containing formamide at a temperature of 42°C. In this case, the concentration of formamide in the hybridization buffer may be reduced in 5% increments from 50% to 0% to identify clones having decreasing levels of homology to the probe. Following hybridization, the filter may be washed with 6X SSC, 0.5% SDS at 50°C. These conditions are considered to be "moderate" conditions above 25% formamide and "low" conditions below 25% formamide. A specific example of "moderate" hybridization conditions is when the above hybridization is conducted at 30% formamide. A specific example of "low stringency" hybridization conditions is when the above hybridization is conducted at 10% formamide.

However, the selection of a hybridization format is not critical - it is the stringency of the wash conditions that set forth the conditions which determine whether a nucleic acid is within the scope of the invention. Wash conditions used to identify nucleic acids within the scope of the invention include, e.g.: a salt concentration of about 0.02 molar at pH 7 and a temperature of at least about 50°C or about 55°C to about 60°C; or, a salt concentration of about 0.15 M NaCl at 72°C for about 15 minutes; or, a salt concentration of about 0.2X SSC at a temperature of at least about 50°C or about 55°C to about 60°C for about 15 to about 20 minutes; or, the hybridization complex is washed twice with a solution with a salt concentration of about 2X SSC containing 0.1% SDS at room temperature for 15 minutes and then washed twice by 0.1X SSC containing 0.1% SDS at 68oC for 15 minutes; or, equivalent conditions. See Sambrook, Tijssen and Ausubel for a description of SSC buffer and equivalent conditions.

These methods may be used to isolate nucleic acids of the invention. For example, the preceding methods may be used to isolate nucleic acids having a sequence with at least about 97%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, or at least 50% homology to a nucleic acid sequence selected from the group consisting of one of the sequences of Group A nucleic acid sequences and sequences substantially identical thereto, or fragments comprising at least about 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive bases thereof and the sequences complementary thereto. Homology may be measured using the alignment algorithm. For example, the homologous polynucleotides may have a coding sequence which is a naturally occurring allelic variant of one of the coding sequences

described herein. Such allelic variants may have a substitution, deletion or addition of one or more nucleotides when compared to the nucleic acids of Group A nucleic acid sequences or the sequences complementary thereto.

Additionally, the above procedures may be used to isolate nucleic acids which encode polypeptides having at least about 99%, 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, or at least 50% homology to a polypeptide having the sequence of one of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof as determined using a sequence alignment algorithm (e.g., such as the FASTA version 3.0t78 algorithm with the default parameters).

Oligonucleotides probes and methods for using them

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The invention also provides nucleic acid probes that can be used, e.g., for identifying nucleic acids encoding a polypeptide with a xylanase activity or fragments thereof or for identifying xylanase genes. In one aspect, the probe comprises at least 10 consecutive bases of a nucleic acid of the invention. Alternatively, a probe of the invention can be at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150 or about 10 to 50, about 20 to 60 about 30 to 70, consecutive bases of a sequence as set forth in a nucleic acid of the invention. The probes identify a nucleic acid by binding and/or hybridization. The probes can be used in arrays of the invention, see discussion below, including, e.g., capillary arrays. The probes of the invention can also be used to isolate other nucleic acids or polypeptides.

The isolated nucleic acids of Group A nucleic acid sequences and sequences substantially identical thereto, the sequences complementary thereto, or a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive bases of one of the sequences of Group A nucleic acid sequences and sequences substantially identical thereto, or the sequences complementary thereto may also be used as probes to determine whether a biological sample, such as a soil sample, contains an organism having a nucleic acid sequence of the invention or an organism from which the nucleic acid was obtained. In such procedures, a biological sample potentially harboring the organism from which the nucleic acid was isolated is obtained and nucleic acids are obtained from the sample. The nucleic acids are contacted with the probe under conditions which permit the probe to specifically hybridize to any complementary sequences from which are present therein.

Where necessary, conditions which permit the probe to specifically hybridize to complementary sequences may be determined by placing the probe in contact with complementary sequences from samples known to contain the complementary sequence as well as control sequences which do not contain the complementary sequence. Hybridization conditions, such as the salt concentration of the hybridization buffer, the formamide concentration of the hybridization buffer, or the hybridization temperature, may be varied to identify conditions which allow the probe to hybridize specifically to complementary nucleic acids.

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If the sample contains the organism from which the nucleic acid was isolated, specific hybridization of the probe is then detected. Hybridization may be detected by labeling the probe with a detectable agent such as a radioactive isotope, a fluorescent dye or an enzyme capable of catalyzing the formation of a detectable product.

Many methods for using the labeled probes to detect the presence of complementary nucleic acids in a sample are familiar to those skilled in the art. These include Southern Blots, Northern Blots, colony hybridization procedures and dot blots. Protocols for each of these procedures are provided in Ausubel et al. Current Protocols in Molecular Biology, John Wiley 503 Sons, Inc. (1997) and Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd Ed., Cold Spring Harbor Laboratory Press (1989.

Alternatively, more than one probe (at least one of which is capable of specifically hybridizing to any complementary sequences which are present in the nucleic acid sample), may be used in an amplification reaction to determine whether the sample contains an organism containing a nucleic acid sequence of the invention (e.g., an organism from which the nucleic acid was isolated). Typically, the probes comprise oligonucleotides. In one aspect, the amplification reaction may comprise a PCR reaction. PCR protocols are described in Ausubel and Sambrook, supra. Alternatively, the amplification may comprise a ligase chain reaction, 3SR, or strand displacement reaction. (See Barany, F., "The Ligase Chain Reaction in a PCR World", PCR Methods and Applications 1:5-16, 1991; E. Fahy et al., "Selfsustained Sequence Replication (3SR): An Isothermal Transcription-based Amplification System Alternative to PCR", PCR Methods and Applications 1:25-33, 1991; and Walker G.T. et al., "Strand Displacement Amplification-an Isothermal in vitro DNA Amplification Technique", Nucleic Acid Research 20:1691-1696, 1992). In such procedures, the nucleic acids in the sample are contacted with the probes, the amplification reaction is performed and any resulting amplification product is detected. The amplification product may be detected by performing gel electrophoresis on the reaction products and staining the gel with an intercalator such as

ethidium bromide. Alternatively, one or more of the probes may be labeled with a radioactive isotope and the presence of a radioactive amplification product may be detected by autoradiography after gel electrophoresis.

Probes derived from sequences near the ends of the sequences of Group A nucleic acid sequences and sequences substantially identical thereto, may also be used in chromosome walking procedures to identify clones containing genomic sequences located adjacent to the sequences of Group A nucleic acid sequences and sequences substantially identical thereto. Such methods allow the isolation of genes which encode additional proteins from the host organism.

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The isolated nucleic acids of Group A nucleic acid sequences and sequences substantially identical thereto, the sequences complementary thereto, or a fragment comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive bases of one of the sequences of Group A nucleic acid sequences and sequences substantially identical thereto, or the sequences complementary thereto may be used as probes to identify and isolate related nucleic acids. In some aspects, the related nucleic acids may be cDNAs or genomic DNAs from organisms other than the one from which the nucleic acid was isolated. For example, the other organisms may be related organisms. In such procedures, a nucleic acid sample is contacted with the probe under conditions which permit the probe to specifically hybridize to related sequences. Hybridization of the probe to nucleic acids from the related organism is then detected using any of the methods described above.

By varying the stringency of the hybridization conditions used to identify nucleic acids, such as cDNAs or genomic DNAs, which hybridize to the detectable probe, nucleic acids having different levels of homology to the probe can be identified and isolated. Stringency may be varied by conducting the hybridization at varying temperatures below the melting temperatures of the probes. The melting temperature, T_m , is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly complementary probe. Very stringent conditions are selected to be equal to or about 5°C lower than the T_m for a particular probe. The melting temperature of the probe may be calculated using the following formulas:

For probes between 14 and 70 nucleotides in length the melting temperature (T_m) is calculated using the formula: $T_m=81.5+16.6(\log [Na+])+0.41(fraction G+C)-(600/N)$ where N is the length of the probe.

If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation: $T_m=81.5+16.6(\log [Na+])+0.41(fraction G+C)-(0.63\% formamide)-(600/N)$ where N is the length of the probe.

Prehybridization may be carried out in 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100µg denatured fragmented salmon sperm DNA or 6X SSC, 5X Denhardt's reagent, 0.5% SDS, 100µg denatured fragmented salmon sperm DNA, 50% formamide. The formulas for SSC and Denhardt's solutions are listed in Sambrook *et al.*, *supra*.

Hybridization is conducted by adding the detectable probe to the prehybridization solutions listed above. Where the probe comprises double stranded DNA, it is denatured before addition to the hybridization solution. The filter is contacted with the hybridization solution for a sufficient period of time to allow the probe to hybridize to cDNAs or genomic DNAs containing sequences complementary thereto or homologous thereto. For probes over 200 nucleotides in length, the hybridization may be carried out at 15-25°C below the T_m. For shorter probes, such as oligonucleotide probes, the hybridization may be conducted at 5-10°C below the T_m. Typically, for hybridizations in 6X SSC, the hybridization is conducted at approximately 68°C. Usually, for hybridizations in 50% formamide containing solutions, the hybridization is conducted at approximately 42°C.

Inhibiting Expression of Xylanases

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The invention provides nucleic acids complementary to (e.g., antisense sequences to) the nucleic acids of the invention, e.g., xylanase-encoding nucleic acids. Antisense sequences are capable of inhibiting the transport, splicing or transcription of xylanase-encoding genes. The inhibition can be effected through the targeting of genomic DNA or messenger RNA. The transcription or function of targeted nucleic acid can be inhibited, for example, by hybridization and/or cleavage. One particularly useful set of inhibitors provided by the present invention includes oligonucleotides which are able to either bind xylanase gene or message, in either case preventing or inhibiting the production or function of xylanase. The association can be through sequence specific hybridization.

Another useful class of inhibitors includes oligonucleotides which cause inactivation or cleavage of xylanase message. The oligonucleotide can have enzyme activity which causes such cleavage, such as ribozymes. The oligonucleotide can be chemically modified or conjugated to an enzyme or composition capable of cleaving the complementary nucleic acid. A pool of many different such oligonucleotides can be screened for those with the desired activity. Thus, the invention provides various compositions for the inhibition of xylanase

expression on a nucleic acid and/or protein level, e.g., antisense, iRNA and ribozymes comprising xylanase sequences of the invention and the anti-xylanase antibodies of the invention.

Inhibition of xylanase expression can have a variety of industrial applications. For example, inhibition of xylanase expression can slow or prevent spoilage. Spoilage can 5 occur when polysaccharides, e.g., structural polysaccharides, are enzymatically degraded. This can lead to the deterioration, or rot, of fruits and vegetables. In one aspect, use of compositions of the invention that inhibit the expression and/or activity of xylanases, e.g., antibodies, antisense oligonucleotides, ribozymes and RNAi, are used to slow or prevent 10 spoilage. Thus, in one aspect, the invention provides methods and compositions comprising application onto a plant or plant product (e.g., a cereal, a grain, a fruit, seed, root, leaf, etc.) antibodies, antisense oligonucleotides, ribozymes and RNAi of the invention to slow or prevent spoilage. These compositions also can be expressed by the plant (e.g., a transgenic plant) or another organism (e.g., a bacterium or other microorganism transformed with a xylanase gene of the invention).

The compositions of the invention for the inhibition of xylanase expression (e.g., antisense, iRNA, ribozymes, antibodies) can be used as pharmaceutical compositions, e.g., as anti-pathogen agents or in other therapies, e.g., as anti-microbials for, e.g., Salmonella.

Antisense Oligonucleotides

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The invention provides antisense oligonucleotides capable of binding xylanase message which can inhibit xylan hydrolase activity (e.g., catalyzing hydrolysis of internal β-1,4-xylosidic linkages) by targeting mRNA. Strategies for designing antisense oligonucleotides are well described in the scientific and patent literature, and the skilled artisan can design such xylanase oligonucleotides using the novel reagents of the invention. For example, gene walking/RNA mapping protocols to screen for effective antisense oligonucleotides are well known in the art, see, e.g., Ho (2000) Methods Enzymol. 314:168-183, describing an RNA mapping assay, which is based on standard molecular techniques to provide an easy and reliable method for potent antisense sequence selection. See also Smith (2000) Eur. J. Pharm. Sci. 11:191-198.

Naturally occurring nucleic acids are used as antisense oligonucleotides. The antisense oligonucleotides can be of any length; for example, in alternative aspects, the antisense oligonucleotides are between about 5 to 100, about 10 to 80, about 15 to 60, about

18 to 40. The optimal length can be determined by routine screening. The antisense oligonucleotides can be present at any concentration. The optimal concentration can be determined by routine screening. A wide variety of synthetic, non-naturally occurring nucleotide and nucleic acid analogues are known which can address this potential problem. For example, peptide nucleic acids (PNAs) containing non-ionic backbones, such as N-(2-

For example, peptide nucleic acids (PNAs) containing non-ionic backbones, such as N-(2-aminoethyl) glycine units can be used. Antisense oligonucleotides having phosphorothioate linkages can also be used, as described in WO 97/03211; WO 96/39154; Mata (1997) Toxicol Appl Pharmacol 144:189-197; Antisense Therapeutics, ed. Agrawal (Humana Press, Totowa, N.J., 1996). Antisense oligonucleotides having synthetic DNA backbone analogues provided by the invention can also include phosphoro-dithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, and morpholino carbamate nucleic acids, as described above.

Combinatorial chemistry methodology can be used to create vast numbers of oligonucleotides that can be rapidly screened for specific oligonucleotides that have appropriate binding affinities and specificities toward any target, such as the sense and antisense xylanase sequences of the invention (see, e.g., Gold (1995) J. of Biol. Chem. 270:13581-13584).

Inhibitory Ribozymes

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These ribozymes can inhibit xylanase activity by, e.g., targeting mRNA. Strategies for designing ribozymes and selecting the xylanase-specific antisense sequence for targeting are well described in the scientific and patent literature, and the skilled artisan can design such ribozymes using the novel reagents of the invention. Ribozymes act by binding to a target RNA through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA that cleaves the target RNA. Thus, the ribozyme recognizes and binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cleave and inactivate the target RNA. Cleavage of a target RNA in such a manner will destroy its ability to direct synthesis of an encoded protein if the cleavage occurs in the coding sequence. After a ribozyme has bound and cleaved its RNA target, it can be released from that RNA to bind and cleave new targets repeatedly.

In some circumstances, the enzymatic nature of a ribozyme can be advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its transcription, translation or

association with another molecule) as the effective concentration of ribozyme necessary to effect a therapeutic treatment can be lower than that of an antisense oligonucleotide. This potential advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, a ribozyme is typically a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ratio of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, the specificity of action of a ribozyme can be greater than that of antisense oligonucleotide binding the same RNA site.

The ribozyme of the invention, e.g., an enzymatic ribozyme RNA molecule, can be formed in a hammerhead motif, a hairpin motif, as a hepatitis delta virus motif, a group I intron motif and/or an RNaseP-like RNA in association with an RNA guide sequence. Examples of hammerhead motifs are described by, e.g., Rossi (1992) Aids Research and Human Retroviruses 8:183; hairpin motifs by Hampel (1989) Biochemistry 28:4929, and Hampel (1990) Nuc. Acids Res. 18:299; the hepatitis delta virus motif by Perrotta (1992) Biochemistry 31:16; the RNaseP motif by Guerrier-Takada (1983) Cell 35:849; and the group I intron by Cech U.S. Pat. No. 4,987,071. The recitation of these specific motifs is not intended to be limiting. Those skilled in the art will recognize that a ribozyme of the invention, e.g., an enzymatic RNA molecule of this invention, can have a specific substrate binding site complementary to one or more of the target gene RNA regions. A ribozyme of the invention can have a nucleotide sequence within or surrounding that substrate binding site which imparts an RNA cleaving activity to the molecule.

RNA interference (RNAi)

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In one aspect, the invention provides an RNA inhibitory molecule, a so-called "RNAi" molecule, comprising a xylanase sequence of the invention. The RNAi molecule comprises a double-stranded RNA (dsRNA) molecule. The RNAi can inhibit expression of a xylanase gene. In one aspect, the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length. While the invention is not limited by any particular mechanism of action, the RNAi can enter a cell and cause the degradation of a single-stranded RNA (ssRNA) of similar or identical sequences, including endogenous mRNAs.

When a cell is exposed to double-stranded RNA (dsRNA), mRNA from the homologous gene is selectively degraded by a process called RNA interference (RNAi). A possible basic mechanism behind RNAi is the breaking of a double-stranded RNA (dsRNA) matching a specific gene sequence into short pieces called short interfering RNA, which trigger the degradation of mRNA that matches its sequence. In one aspect, the RNAi's of the invention are used in gene-silencing therapeutics, see, e.g., Shuey (2002) Drug Discov. Today 7:1040-1046. In one aspect, the invention provides methods to selectively degrade RNA using the RNAi's of the invention. The process may be practiced *in vitro*, ex vivo or *in vivo*. In one aspect, the RNAi molecules of the invention can be used to generate a loss-of-function mutation in a cell, an organ or an animal. Methods for making and using RNAi molecules for selectively degrade RNA are well known in the art, see, e.g., U.S. Patent No. 6,506,559; 6,511,824; 6,515,109; 6,489,127.

Modification of Nucleic Acids

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The invention provides methods of generating variants of the nucleic acids of the invention, e.g., those encoding a xylanase. These methods can be repeated or used in various combinations to generate xylanases having an altered or different activity or an altered or different stability from that of a xylanase encoded by the template nucleic acid. These methods also can be repeated or used in various combinations, e.g., to generate variations in gene/ message expression, message translation or message stability. In another aspect, the genetic composition of a cell is altered by, e.g., modification of a homologous gene ex vivo, followed by its reinsertion into the cell.

A nucleic acid of the invention can be altered by any means. For example, random or stochastic methods, or, non-stochastic, or "directed evolution," methods, see, e.g., U.S. Patent No. 6,361,974. Methods for random mutation of genes are well known in the art, see, e.g., U.S. Patent No. 5,830,696. For example, mutagens can be used to randomly mutate a gene. Mutagens include, e.g., ultraviolet light or gamma irradiation, or a chemical mutagen, e.g., mitomycin, nitrous acid, photoactivated psoralens, alone or in combination, to induce DNA breaks amenable to repair by recombination. Other chemical mutagens include, for example, sodium bisulfite, nitrous acid, hydroxylamine, hydrazine or formic acid. Other mutagens are analogues of nucleotide precursors, e.g., nitrosoguanidine, 5-bromouracil, 2-aminopurine, or acridine. These agents can be added to a PCR reaction in place of the nucleotide precursor thereby mutating the sequence. Intercalating agents such as proflavine, acriflavine, quinacrine and the like can also be used.

Any technique in molecular biology can be used, e.g., random PCR mutagenesis, see, e.g., Rice (1992) Proc. Natl. Acad. Sci. USA 89:5467-5471; or, combinatorial multiple cassette mutagenesis, see, e.g., Crameri (1995) Biotechniques 18:194-196. Alternatively, nucleic acids, e.g., genes, can be reassembled after random, or "stochastic," fragmentation, see, e.g., U.S. Patent Nos. 6,291,242; 6,287,862; 6,287,861; 5 5,955,358; 5,830,721; 5,824,514; 5,811,238; 5,605,793. In alternative aspects, modifications, additions or deletions are introduced by error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-10 specific mutagenesis, gene reassembly (e.g., GeneReassembly™, see, e.g., U.S. Patent No. 6,537,776), gene site saturated mutagenesis (GSSMTM), synthetic ligation reassembly (SLR), recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-15 purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation, and/or a combination of these and other methods.

The following publications describe a variety of recursive recombination procedures and/or methods which can be incorporated into the methods of the invention: 20 Stemmer (1999) "Molecular breeding of viruses for targeting and other clinical properties" Tumor Targeting 4:1-4; Ness (1999) Nature Biotechnology 17:893-896; Chang (1999) "Evolution of a cytokine using DNA family shuffling" Nature Biotechnology 17:793-797; Minshull (1999) "Protein evolution by molecular breeding" Current Opinion in Chemical Biology 3:284-290; Christians (1999) "Directed evolution of thymidine kinase for AZT 25 phosphorylation using DNA family shuffling" Nature Biotechnology 17:259-264; Crameri (1998) "DNA shuffling of a family of genes from diverse species accelerates directed evolution" Nature 391:288-291; Crameri (1997) "Molecular evolution of an arsenate detoxification pathway by DNA shuffling," Nature Biotechnology 15:436-438; Zhang (1997) "Directed evolution of an effective fucosidase from a galactosidase by DNA shuffling and 30 screening" Proc. Natl. Acad. Sci. USA 94:4504-4509; Pattern et al. (1997) "Applications of DNA Shuffling to Pharmaceuticals and Vaccines" Current Opinion in Biotechnology 8:724 733; Crameri et al. (1996) "Construction and evolution of antibody-phage libraries by DNA shuffling" Nature Medicine 2:100-103; Gates et al. (1996) "Affinity selective isolation of ligands from peptide libraries through display on a lac repressor 'headpiece dimer'" Journal

of Molecular Biology 255:373-386; Stemmer (1996) "Sexual PCR and Assembly PCR" In: The Encyclopedia of Molecular Biology. VCH Publishers, New York. pp.447-457; Crameri and Stemmer (1995) "Combinatorial multiple cassette mutagenesis creates all the permutations of mutant and wildtype cassettes" BioTechniques 18:194-195; Stemmer et al. (1995) "Single-step assembly of a gene and entire plasmid form large numbers of

oligodeoxyribonucleotides" Gene, 164:49-53; Stemmer (1995) "The Evolution of Molecular Computation" Science 270: 1510; Stemmer (1995) "Searching Sequence Space" Bio/Technology 13:549-553; Stemmer (1994) "Rapid evolution of a protein in vitro by DNA shuffling" Nature 370:389-391; and Stemmer (1994) "DNA shuffling by random fragmentation and reassembly: In vitro recombination for molecular evolution." Proc. Natl.

Acad. Sci. USA 91:10747-10751.

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Mutational methods of generating diversity include, for example, site-directed mutagenesis (Ling et al. (1997) "Approaches to DNA mutagenesis: an overview" Anal Biochem. 254(2): 157-178; Dale et al. (1996) "Oligonucleotide-directed random mutagenesis 15 using the phosphorothioate method" Methods Mol. Biol. 57:369-374; Smith (1985) "In vitro mutagenesis" Ann. Rev. Genet. 19:423-462; Botstein & Shortle (1985) "Strategies and applications of in vitro mutagenesis" Science 229:1193-1201; Carter (1986) "Site-directed mutagenesis" Biochem. J. 237:1-7; and Kunkel (1987) "The efficiency of oligonucleotide directed mutagenesis" in Nucleic Acids & Molecular Biology (Eckstein, F. and Lilley, D. M. 20 J. eds., Springer Verlag, Berlin)); mutagenesis using uracil containing templates (Kunkel (1985) "Rapid and efficient site-specific mutagenesis without phenotypic selection" Proc. Natl. Acad. Sci. USA 82:488-492; Kunkel et al. (1987) "Rapid and efficient site-specific mutagenesis without phenotypic selection" Methods in Enzymol. 154, 367-382; and Bass et al. (1988) "Mutant Trp repressors with new DNA-binding specificities" Science 242:240-25 245); oligonucleotide-directed mutagenesis (Methods in Enzymol. 100: 468-500 (1983); Methods in Enzymol. 154: 329-350 (1987); Zoller (1982) "Oligonucleotide-directed mutagenesis using M13-derived vectors: an efficient and general procedure for the production of point mutations in any DNA fragment" Nucleic Acids Res. 10:6487-6500; Zoller & Smith (1983) "Oligonucleotide-directed mutagenesis of DNA fragments cloned into M13 vectors" Methods in Enzymol. 100:468-500; and Zoller (1987) Oligonucleotide-directed 30 mutagenesis: a simple method using two oligonucleotide primers and a single-stranded DNA template" Methods in Enzymol. 154:329-350); phosphorothioate-modified DNA mutagenesis (Taylor (1985) "The use of phosphorothioate-modified DNA in restriction enzyme reactions to prepare nicked DNA" Nucl. Acids Res. 13: 8749-8764; Taylor (1985) "The rapid

generation of oligonucleotide-directed mutations at high frequency using phosphorothioate-modified DNA" Nucl. Acids Res. 13: 8765-8787 (1985); Nakamaye (1986) "Inhibition of restriction endonuclease Nci I cleavage by phosphorothioate groups and its application to oligonucleotide-directed mutagenesis" Nucl. Acids Res. 14: 9679-9698; Sayers (1988) "Y-T Exonucleases in phosphorothioate-based oligonucleotide-directed mutagenesis" Nucl. Acids Res. 16:791-802; and Sayers et al. (1988) "Strand specific cleavage of phosphorothioate-containing DNA by reaction with restriction endonucleases in the presence of ethidium bromide" Nucl. Acids Res. 16: 803-814); mutagenesis using gapped duplex DNA (Kramer et al. (1984) "The gapped duplex DNA approach to oligonucleotide-directed mutation construction" Nucl. Acids Res. 12: 9441-9456; Kramer & Fritz (1987) Methods in Enzymol. "Oligonucleotide-directed construction of mutations via gapped duplex DNA" 154:350-367; Kramer (1988) "Improved enzymatic in vitro reactions in the gapped duplex DNA approach to oligonucleotide-directed construction of mutations" Nucl. Acids Res. 16: 7207; and Fritz (1988) "Oligonucleotide-directed construction of mutations: a gapped duplex DNA procedure without enzymatic reactions in vitro" Nucl. Acids Res. 16: 6987-6999).

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Additional protocols that can be used to practice the invention include point mismatch repair (Kramer (1984) "Point Mismatch Repair" Cell 38:879-887), mutagenesis using repair-deficient host strains (Carter et al. (1985) "Improved oligonucleotide sitedirected mutagenesis using M13 vectors" Nucl. Acids Res. 13: 4431-4443; and Carter (1987) "Improved oligonucleotide-directed mutagenesis using M13 vectors" Methods in Enzymol. 154: 382-403), deletion mutagenesis (Eghtedarzadeh (1986) "Use of oligonucleotides to generate large deletions" Nucl. Acids Res. 14: 5115), restriction-selection and restrictionselection and restriction-purification (Wells et al. (1986) "Importance of hydrogen-bond formation in stabilizing the transition state of subtilisin" Phil. Trans. R. Soc. Lond. A 317: 415-423), mutagenesis by total gene synthesis (Nambiar et al. (1984) "Total synthesis and cloning of a gene coding for the ribonuclease S protein" Science 223: 1299-1301; Sakamar and Khorana (1988) "Total synthesis and expression of a gene for the a-subunit of bovine rod outer segment guanine nucleotide-binding protein (transducin)" Nucl. Acids Res. 14: 6361-6372; Wells et al. (1985) "Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites" Gene 34:315-323; and Grundstrom et al. (1985) "Oligonucleotide-directed mutagenesis by microscale `shot-gun` gene synthesis" Nucl. Acids Res. 13: 3305-3316), double-strand break repair (Mandecki (1986); Arnold (1993) "Protein engineering for unusual environments" Current Opinion in Biotechnology 4:450-455. "Oligonucleotide-directed double-strand break repair in plasmids of Escherichia coli: a

method for site-specific mutagenesis" Proc. Natl. Acad. Sci. USA, 83:7177-7181). Additional details on many of the above methods can be found in Methods in Enzymology Volume 154, which also describes useful controls for trouble-shooting problems with various mutagenesis methods.

5 Protocols that can be used to practice the invention are described, e.g., in U.S. Patent Nos. 5,605,793 to Stemmer (Feb. 25, 1997), "Methods for In Vitro Recombination;" U.S. Pat. No. 5,811,238 to Stemmer et al. (Sep. 22, 1998) "Methods for Generating Polynucleotides having Desired Characteristics by Iterative Selection and Recombination;" U.S. Pat. No. 5,830,721 to Stemmer et al. (Nov. 3, 1998), "DNA Mutagenesis by Random 10 Fragmentation and Reassembly;" U.S. Pat. No. 5,834,252 to Stemmer, et al. (Nov. 10, 1998) "End-Complementary Polymerase Reaction;" U.S. Pat. No. 5,837,458 to Minshull, et al. (Nov. 17, 1998), "Methods and Compositions for Cellular and Metabolic Engineering;" WO 95/22625, Stemmer and Crameri, "Mutagenesis by Random Fragmentation and Reassembly;" WO 96/33207 by Stemmer and Lipschutz "End Complementary Polymerase Chain 15 Reaction;" WO 97/20078 by Stemmer and Crameri "Methods for Generating Polynucleotides having Desired Characteristics by Iterative Selection and Recombination;" WO 97/35966 by Minshull and Stemmer, "Methods and Compositions for Cellular and Metabolic Engineering;" WO 99/41402 by Punnonen et al. "Targeting of Genetic Vaccine Vectors;" WO 99/41383 by Punnonen et al. "Antigen Library Immunization;" WO 99/41369 by 20 Punnonen et al. "Genetic Vaccine Vector Engineering:" WO 99/41368 by Punnonen et al. "Optimization of Immunomodulatory Properties of Genetic Vaccines;" EP 752008 by Stemmer and Crameri, "DNA Mutagenesis by Random Fragmentation and Reassembly;" EP 0932670 by Stemmer "Evolving Cellular DNA Uptake by Recursive Sequence Recombination;" WO 99/23107 by Stemmer et al., "Modification of Virus Tropism and Host 25 Range by Viral Genome Shuffling;" WO 99/21979 by Apt et al., "Human Papillomavirus Vectors;" WO 98/31837 by del Cardayre et al. "Evolution of Whole Cells and Organisms by Recursive Sequence Recombination;" WO 98/27230 by Pattern and Stemmer, "Methods and Compositions for Polypeptide Engineering;" WO 98/27230 by Stemmer et al., "Methods for Optimization of Gene Therapy by Recursive Sequence Shuffling and Selection," WO 30 00/00632, "Methods for Generating Highly Diverse Libraries," WO 00/09679, "Methods for Obtaining in Vitro Recombined Polynucleotide Sequence Banks and Resulting Sequences," WO 98/42832 by Arnold et al., "Recombination of Polynucleotide Sequences Using Random or Defined Primers," WO 99/29902 by Arnold et al., "Method for Creating Polynucleotide and Polypeptide Sequences," WO 98/41653 by Vind, "An in Vitro Method for Construction

of a DNA Library," WO 98/41622 by Borchert et al., "Method for Constructing a Library Using DNA Shuffling," and WO 98/42727 by Pati and Zarling, "Sequence Alterations using Homologous Recombination."

Protocols that can be used to practice the invention (providing details regarding various diversity generating methods) are described, e.g., in U.S. Patent application 5 serial no. (USSN) 09/407,800, "SHUFFLING OF CODON ALTERED GENES" by Patten et al. filed Sep. 28, 1999; "EVOLUTION OF WHOLE CELLS AND ORGANISMS BY RECURSIVE SEQUENCE RECOMBINATION" by del Cardayre et al., United States Patent No. 6,379,964; "OLIGONUCLEOTIDE MEDIATED NUCLEIC ACID RECOMBINATION" by Crameri et al., United States Patent Nos. 6,319,714; 6,368,861; 10 6,376,246; 6,423,542; 6,426,224 and PCT/US00/01203; "USE OF CODON-VARIED OLIGONUCLEOTIDE SYNTHESIS FOR SYNTHETIC SHUFFLING" by Welch et al., United States Patent No. 6,436,675; "METHODS FOR MAKING CHARACTER STRINGS, POLYNUCLEOTIDES & POLYPEPTIDES HAVING DESIRED CHARACTERISTICS" by Selifonov et al., filed Jan. 18, 2000, (PCT/US00/01202) and, e.g. "METHODS FOR 15 MAKING CHARACTER STRINGS, POLYNUCLEOTIDES & POLYPEPTIDES HAVING DESIRED CHARACTERISTICS" by Selifonov et al., filed Jul. 18, 2000 (U.S. Ser. No. 09/618,579); "METHODS OF POPULATING DATA STRUCTURES FOR USE IN EVOLUTIONARY SIMULATIONS" by Selifonov and Stemmer, filed Jan. 18, 2000 (PCT/US00/01138); and "SINGLE-STRANDED NUCLEIC ACID TEMPLATE-20 MEDIATED RECOMBINATION AND NUCLEIC ACID FRAGMENT ISOLATION" by Affholter, filed Sep. 6, 2000 (U.S. Ser. No. 09/656,549); and United States Patent Nos. 6,177,263; 6,153,410.

Non-stochastic, or "directed evolution," methods include, e.g., saturation mutagenesis (GSSMTM), synthetic ligation reassembly (SLR), or a combination thereof are used to modify the nucleic acids of the invention to generate xylanases with new or altered properties (e.g., activity under highly acidic or alkaline conditions, high or low temperatures, and the like). Polypeptides encoded by the modified nucleic acids can be screened for an activity before testing for xylan hydrolysis or other activity. Any testing modality or protocol can be used, e.g., using a capillary array platform. See, e.g., U.S. Patent Nos. 6,361,974; 6,280,926; 5,939,250.

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Saturation mutagenesis, or, GSSMTM

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In one aspect, codon primers containing a degenerate N,N,G/T sequence are used to introduce point mutations into a polynucleotide, e.g., a xylanase or an antibody of the invention, so as to generate a set of progeny polypeptides in which a full range of single amino acid substitutions is represented at each amino acid position, e.g., an amino acid residue in an enzyme active site or ligand binding site targeted to be modified. These oligonucleotides can comprise a contiguous first homologous sequence, a degenerate N,N,G/T sequence, and, optionally, a second homologous sequence. The downstream progeny translational products from the use of such oligonucleotides include all possible amino acid changes at each amino acid site along the polypeptide, because the degeneracy of the N,N,G/T sequence includes codons for all 20 amino acids. In one aspect, one such degenerate oligonucleotide (comprised of, e.g., one degenerate N,N,G/T cassette) is used for subjecting each original codon in a parental polynucleotide template to a full range of codon substitutions. In another aspect, at least two degenerate cassettes are used - either in the same oligonucleotide or not, for subjecting at least two original codons in a parental polynucleotide template to a full range of codon substitutions. For example, more than one N,N,G/T sequence can be contained in one oligonucleotide to introduce amino acid mutations at more than one site. This plurality of N,N,G/T sequences can be directly contiguous, or separated by one or more additional nucleotide sequence(s). In another aspect, oligonucleotides serviceable for introducing additions and deletions can be used either alone or in combination with the codons containing an N,N,G/T sequence, to introduce any combination or permutation of amino acid additions, deletions, and/or substitutions.

In one aspect, simultaneous mutagenesis of two or more contiguous amino acid positions is done using an oligonucleotide that contains contiguous N,N,G/T triplets, i.e. a degenerate (N,N,G/T)n sequence. In another aspect, degenerate cassettes having less degeneracy than the N,N,G/T sequence are used. For example, it may be desirable in some instances to use (e.g. in an oligonucleotide) a degenerate triplet sequence comprised of only one N, where said N can be in the first second or third position of the triplet. Any other bases including any combinations and permutations thereof can be used in the remaining two positions of the triplet. Alternatively, it may be desirable in some instances to use (e.g. in an oligo) a degenerate N,N,N triplet sequence.

In one aspect, use of degenerate triplets (e.g., N,N,G/T triplets) allows for systematic and easy generation of a full range of possible natural amino acids (for a total of 20 amino acids) into each and every amino acid position in a polypeptide (in alternative

aspects, the methods also include generation of less than all possible substitutions per amino acid residue, or codon, position). For example, for a 100 amino acid polypeptide, 2000 distinct species (i.e. 20 possible amino acids per position X 100 amino acid positions) can be generated. Through the use of an oligonucleotide or set of oligonucleotides containing a degenerate N,N,G/T triplet, 32 individual sequences can code for all 20 possible natural amino acids. Thus, in a reaction vessel in which a parental polynucleotide sequence is subjected to saturation mutagenesis using at least one such oligonucleotide, there are generated 32 distinct progeny polynucleotides encoding 20 distinct polypeptides. In contrast, the use of a non-degenerate oligonucleotide in site-directed mutagenesis leads to only one progeny polypeptide product per reaction vessel. Nondegenerate oligonucleotides can optionally be used in combination with degenerate primers disclosed; for example, nondegenerate oligonucleotides can be used to generate specific point mutations in a working polynucleotide. This provides one means to generate specific silent point mutations, point mutations leading to corresponding amino acid changes, and point mutations that cause the generation of stop codons and the corresponding expression of polypeptide fragments.

In one aspect, each saturation mutagenesis reaction vessel contains polynucleotides encoding at least 20 progeny polypeptide (e.g., xylanases) molecules such that all 20 natural amino acids are represented at the one specific amino acid position corresponding to the codon position mutagenized in the parental polynucleotide (other aspects use less than all 20 natural combinations). The 32-fold degenerate progeny polypeptides generated from each saturation mutagenesis reaction vessel can be subjected to clonal amplification (e.g. cloned into a suitable host, e.g., *E. coli* host, using, e.g., an expression vector) and subjected to expression screening. When an individual progeny polypeptide is identified by screening to display a favorable change in property (when compared to the parental polypeptide, such as increased xylan hydrolysis activity under alkaline or acidic conditions), it can be sequenced to identify the correspondingly favorable amino acid substitution contained therein.

In one aspect, upon mutagenizing each and every amino acid position in a parental polypeptide using saturation mutagenesis as disclosed herein, favorable amino acid changes may be identified at more than one amino acid position. One or more new progeny molecules can be generated that contain a combination of all or part of these favorable amino acid substitutions. For example, if 2 specific favorable amino acid changes are identified in each of 3 amino acid positions in a polypeptide, the permutations include 3 possibilities at each position (no change from the original amino acid, and each of two favorable changes)

and 3 positions. Thus, there are 3 x 3 x 3 or 27 total possibilities, including 7 that were previously examined - 6 single point mutations (i.e. 2 at each of three positions) and no change at any position.

In yet another aspect, site-saturation mutagenesis can be used together with shuffling, chimerization, recombination and other mutagenizing processes, along with screening. This invention provides for the use of any mutagenizing process(es), including saturation mutagenesis, in an iterative manner. In one exemplification, the iterative use of any mutagenizing process(es) is used in combination with screening.

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The invention also provides for the use of proprietary codon primers (containing a degenerate N,N,N sequence) to introduce point mutations into a polynucleotide, so as to generate a set of progeny polypeptides in which a full range of single amino acid substitutions is represented at each amino acid position (gene site saturated mutagenesis (GSSMTM)). The oligos used are comprised contiguously of a first homologous sequence, a degenerate N,N,N sequence and preferably but not necessarily a second homologous sequence. The downstream progeny translational products from the use of such oligos include all possible amino acid changes at each amino acid site along the polypeptide, because the degeneracy of the N,N,N sequence includes codons for all 20 amino acids.

In one aspect, one such degenerate oligo (comprised of one degenerate N,N,N cassette) is used for subjecting each original codon in a parental polynucleotide template to a full range of codon substitutions. In another aspect, at least two degenerate N,N,N cassettes are used – either in the same oligo or not, for subjecting at least two original codons in a parental polynucleotide template to a full range of codon substitutions. Thus, more than one N,N,N sequence can be contained in one oligo to introduce amino acid mutations at more than one site. This plurality of N,N,N sequences can be directly contiguous, or separated by one or more additional nucleotide sequence(s). In another aspect, oligos serviceable for introducing additions and deletions can be used either alone or in combination with the codons containing an N,N,N sequence, to introduce any combination or permutation of amino acid additions, deletions and/or substitutions.

In a particular exemplification, it is possible to simultaneously mutagenize two or more contiguous amino acid positions using an oligo that contains contiguous N,N,N triplets, *i.e.* a degenerate (N,N,N)_n sequence.

In another aspect, the present invention provides for the use of degenerate cassettes having less degeneracy than the N,N,N sequence. For example, it may be desirable in some instances to use (e.g. in an oligo) a degenerate triplet sequence comprised of only one

N, where the N can be in the first second or third position of the triplet. Any other bases including any combinations and permutations thereof can be used in the remaining two positions of the triplet. Alternatively, it may be desirable in some instances to use (e.g., in an oligo) a degenerate N,N,N triplet sequence, N,N,G/T, or an N,N, G/C triplet sequence.

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It is appreciated, however, that the use of a degenerate triplet (such as N,N,G/T or an N,N, G/C triplet sequence) as disclosed in the instant invention is advantageous for several reasons. In one aspect, this invention provides a means to systematically and fairly easily generate the substitution of the full range of possible amino acids (for a total of 20 amino acids) into each and every amino acid position in a polypeptide. Thus, for a 100 amino acid polypeptide, the invention provides a way to systematically and fairly easily generate 2000 distinct species (*i.e.*, 20 possible amino acids per position times 100 amino acid positions). It is appreciated that there is provided, through the use of an oligo containing a degenerate N,N,G/T or an N,N, G/C triplet sequence, 32 individual sequences that code for 20 possible amino acids. Thus, in a reaction vessel in which a parental polynucleotide sequence is subjected to saturation mutagenesis using one such oligo, there are generated 32 distinct progeny polynucleotides encoding 20 distinct polypeptides. In contrast, the use of a non-degenerate oligo in site-directed mutagenesis leads to only one progeny polypeptide product per reaction vessel.

This invention also provides for the use of nondegenerate oligos, which can optionally be used in combination with degenerate primers disclosed. It is appreciated that in some situations, it is advantageous to use nondegenerate oligos to generate specific point mutations in a working polynucleotide. This provides a means to generate specific silent point mutations, point mutations leading to corresponding amino acid changes and point mutations that cause the generation of stop codons and the corresponding expression of polypeptide fragments.

Thus, in one aspect of this invention, each saturation mutagenesis reaction vessel contains polynucleotides encoding at least 20 progeny polypeptide molecules such that all 20 amino acids are represented at the one specific amino acid position corresponding to the codon position mutagenized in the parental polynucleotide. The 32-fold degenerate progeny polypeptides generated from each saturation mutagenesis reaction vessel can be subjected to clonal amplification (e.g., cloned into a suitable E. coli host using an expression vector) and subjected to expression screening. When an individual progeny polypeptide is identified by screening to display a favorable change in property (when compared to the

parental polypeptide), it can be sequenced to identify the correspondingly favorable amino acid substitution contained therein.

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It is appreciated that upon mutagenizing each and every amino acid position in a parental polypeptide using saturation mutagenesis as disclosed herein, favorable amino acid changes may be identified at more than one amino acid position. One or more new progeny molecules can be generated that contain a combination of all or part of these favorable amino acid substitutions. For example, if 2 specific favorable amino acid changes are identified in each of 3 amino acid positions in a polypeptide, the permutations include 3 possibilities at each position (no change from the original amino acid and each of two favorable changes) and 3 positions. Thus, there are 3 x 3 x 3 or 27 total possibilities, including 7 that were previously examined - 6 single point mutations (i.e., 2 at each of three positions) and no change at any position.

Thus, in a non-limiting exemplification, this invention provides for the use of saturation mutagenesis in combination with additional mutagenization processes, such as process where two or more related polynucleotides are introduced into a suitable host cell such that a hybrid polynucleotide is generated by recombination and reductive reassortment.

In addition to performing mutagenesis along the entire sequence of a gene, the instant invention provides that mutagenesis can be use to replace each of any number of bases in a polynucleotide sequence, wherein the number of bases to be mutagenized is preferably every integer from 15 to 100,000. Thus, instead of mutagenizing every position along a molecule, one can subject every or a discrete number of bases (preferably a subset totaling from 15 to 100,000) to mutagenesis. Preferably, a separate nucleotide is used for mutagenizing each position or group of positions along a polynucleotide sequence. A group of 3 positions to be mutagenized may be a codon. The mutations are preferably introduced using a mutagenic primer, containing a heterologous cassette, also referred to as a mutagenic cassette. Exemplary cassettes can have from 1 to 500 bases. Each nucleotide position in such heterologous cassettes be N, A, C, G, T, A/C, A/G, A/T, C/G, C/T, G/T, C/G/T, A/G/T, A/C/T, A/C/G, or E, where E is any base that is not A, C, G, or T (E can be referred to as a designer oligo).

In a general sense, saturation mutagenesis is comprised of mutagenizing a complete set of mutagenic cassettes (wherein each cassette is preferably about 1-500 bases in length) in defined polynucleotide sequence to be mutagenized (wherein the sequence to be mutagenized is preferably from about 15 to 100,000 bases in length). Thus, a group of mutations (ranging from 1 to 100 mutations) is introduced into each cassette to be

mutagenized. A grouping of mutations to be introduced into one cassette can be different or the same from a second grouping of mutations to be introduced into a second cassette during the application of one round of saturation mutagenesis. Such groupings are exemplified by deletions, additions, groupings of particular codons and groupings of particular nucleotide cassettes.

Defined sequences to be mutagenized include a whole gene, pathway, cDNA, an entire open reading frame (ORF) and entire promoter, enhancer, repressor/transactivator, origin of replication, intron, operator, or any polynucleotide functional group. Generally, a "defined sequences" for this purpose may be any polynucleotide that a 15 base-polynucleotide sequence and polynucleotide sequences of lengths between 15 bases and 15,000 bases (this invention specifically names every integer in between). Considerations in choosing groupings of codons include types of amino acids encoded by a degenerate mutagenic cassette.

In one exemplification a grouping of mutations that can be introduced into a mutagenic cassette, this invention specifically provides for degenerate codon substitutions (using degenerate oligos) that code for 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 amino acids at each position and a library of polypeptides encoded thereby.

Synthetic Ligation Reassembly (SLR)

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The invention provides a non-stochastic gene modification system termed "synthetic ligation reassembly," or simply "SLR," a "directed evolution process," to generate polypeptides, e.g., xylanases or antibodies of the invention, with new or altered properties.

SLR is a method of ligating oligonucleotide fragments together non-stochastically. This method differs from stochastic oligonucleotide shuffling in that the nucleic acid building blocks are not shuffled, concatenated or chimerized randomly, but rather are assembled non-stochastically. See, e.g., U.S. Patent Application Serial No. (USSN) 09/332,835 entitled "Synthetic Ligation Reassembly in Directed Evolution" and filed on June 14, 1999 ("USSN 09/332,835"). In one aspect, SLR comprises the following steps: (a) providing a template polynucleotide, wherein the template polynucleotide comprises sequence encoding a homologous gene; (b) providing a plurality of building block polynucleotides, wherein the building block polynucleotides are designed to cross-over reassemble with the template polynucleotide at a predetermined sequence, and a building block polynucleotide comprises a sequence that is a variant of the homologous gene and a sequence homologous to the template polynucleotide flanking the variant sequence; (c)

combining a building block polynucleotide with a template polynucleotide such that the building block polynucleotide cross-over reassembles with the template polynucleotide to generate polynucleotides comprising homologous gene sequence variations.

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SLR does not depend on the presence of high levels of homology between polynucleotides to be rearranged. Thus, this method can be used to non-stochastically generate libraries (or sets) of progeny molecules comprised of over 10¹⁰⁰ different chimeras. SLR can be used to generate libraries comprised of over 10¹⁰⁰⁰ different progeny chimeras. Thus, aspects of the present invention include non-stochastic methods of producing a set of finalized chimeric nucleic acid molecule shaving an overall assembly order that is chosen by design. This method includes the steps of generating by design a plurality of specific nucleic acid building blocks having serviceable mutually compatible ligatable ends, and assembling these nucleic acid building blocks, such that a designed overall assembly order is achieved.

The mutually compatible ligatable ends of the nucleic acid building blocks to be assembled are considered to be "serviceable" for this type of ordered assembly if they enable the building blocks to be coupled in predetermined orders. Thus, the overall assembly order in which the nucleic acid building blocks can be coupled is specified by the design of the ligatable ends. If more than one assembly step is to be used, then the overall assembly order in which the nucleic acid building blocks can be coupled is also specified by the sequential order of the assembly step(s). In one aspect, the annealed building pieces are treated with an enzyme, such as a ligase (e.g. T4 DNA ligase), to achieve covalent bonding of the building pieces.

In one aspect, the design of the oligonucleotide building blocks is obtained by analyzing a set of progenitor nucleic acid sequence templates that serve as a basis for producing a progeny set of finalized chimeric polynucleotides. These parental oligonucleotide templates thus serve as a source of sequence information that aids in the design of the nucleic acid building blocks that are to be mutagenized, e.g., chimerized or shuffled. In one aspect of this method, the sequences of a plurality of parental nucleic acid templates are aligned in order to select one or more demarcation points. The demarcation points can be located at an area of homology, and are comprised of one or more nucleotides. These demarcation points are preferably shared by at least two of the progenitor templates. The demarcation points can thereby be used to delineate the boundaries of oligonucleotide building blocks to be generated in order to rearrange the parental polynucleotides. The demarcation points identified and selected in the progenitor molecules serve as potential chimerization points in the assembly of the final chimeric progeny molecules. A demarcation

point can be an area of homology (comprised of at least one homologous nucleotide base) shared by at least two parental polynucleotide sequences. Alternatively, a demarcation point can be an area of homology that is shared by at least half of the parental polynucleotide sequences, or, it can be an area of homology that is shared by at least two thirds of the parental polynucleotide sequences. Even more preferably a serviceable demarcation points is an area of homology that is shared by at least three fourths of the parental polynucleotide sequences, or, it can be shared by at almost all of the parental polynucleotide sequences. In one aspect, a demarcation point is an area of homology that is shared by all of the parental polynucleotide sequences.

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In one aspect, a ligation reassembly process is performed exhaustively in order to generate an exhaustive library of progeny chimeric polynucleotides. In other words, all possible ordered combinations of the nucleic acid building blocks are represented in the set of finalized chimeric nucleic acid molecules. At the same time, in another aspect, the assembly order (i.e. the order of assembly of each building block in the 5' to 3 sequence of each finalized chimeric nucleic acid) in each combination is by design (or non-stochastic) as described above. Because of the non-stochastic nature of this invention, the possibility of unwanted side products is greatly reduced.

In another aspect, the ligation reassembly method is performed systematically. For example, the method is performed in order to generate a systematically compartmentalized library of progeny molecules, with compartments that can be screened systematically, e.g. one by one. In other words this invention provides that, through the selective and judicious use of specific nucleic acid building blocks, coupled with the selective and judicious use of sequentially stepped assembly reactions, a design can be achieved where specific sets of progeny products are made in each of several reaction vessels. This allows a systematic examination and screening procedure to be performed. Thus, these methods allow a potentially very large number of progeny molecules to be examined systematically in smaller groups. Because of its ability to perform chimerizations in a manner that is highly flexible yet exhaustive and systematic as well, particularly when there is a low level of homology among the progenitor molecules, these methods provide for the generation of a library (or set) comprised of a large number of progeny molecules. Because of the nonstochastic nature of the instant ligation reassembly invention, the progeny molecules generated preferably comprise a library of finalized chimeric nucleic acid molecules having an overall assembly order that is chosen by design. The saturation mutagenesis and optimized directed evolution methods also can be used to generate different progeny

molecular species. It is appreciated that the invention provides freedom of choice and control regarding the selection of demarcation points, the size and number of the nucleic acid building blocks, and the size and design of the couplings. It is appreciated, furthermore, that the requirement for intermolecular homology is highly relaxed for the operability of this invention. In fact, demarcation points can even be chosen in areas of little or no intermolecular homology. For example, because of codon wobble, i.e. the degeneracy of codons, nucleotide substitutions can be introduced into nucleic acid building blocks without altering the amino acid originally encoded in the corresponding progenitor template.

Alternatively, a codon can be altered such that the coding for an originally amino acid is altered. This invention provides that such substitutions can be introduced into the nucleic acid building block in order to increase the incidence of intermolecular homologous demarcation points and thus to allow an increased number of couplings to be achieved among the building blocks, which in turn allows a greater number of progeny chimeric molecules to be generated.

Synthetic gene reassembly

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In one aspect, the present invention provides a non-stochastic method termed synthetic gene reassembly (e.g., GeneReassemblyTM, see, e.g., U.S. Patent No. 6,537,776), which differs from stochastic shuffling in that the nucleic acid building blocks are not shuffled or concatenated or chimerized randomly, but rather are assembled non-stochastically.

The synthetic gene reassembly method does not depend on the presence of a high level of homology between polynucleotides to be shuffled. The invention can be used to non-stochastically generate libraries (or sets) of progeny molecules comprised of over 10¹⁰⁰ different chimeras. Conceivably, synthetic gene reassembly can even be used to generate libraries comprised of over 10¹⁰⁰⁰ different progeny chimeras.

Thus, in one aspect, the invention provides a non-stochastic method of producing a set of finalized chimeric nucleic acid molecules having an overall assembly order that is chosen by design, which method is comprised of the steps of generating by design a plurality of specific nucleic acid building blocks having serviceable mutually compatible ligatable ends and assembling these nucleic acid building blocks, such that a designed overall assembly order is achieved.

In one aspect, synthetic gene reassembly comprises a method of: 1) preparing a progeny generation of molecule(s) (including a molecule comprising a polynucleotide

sequence, e.g., a molecule comprising a polypeptide coding sequence), that is mutagenized to achieve at least one point mutation, addition, deletion, &/or chimerization, from one or more ancestral or parental generation template(s); 2) screening the progeny generation molecule(s), e.g., using a high throughput method, for at least one property of interest (such as an improvement in an enzyme activity); 3) optionally obtaining &/or cataloguing structural &/or and functional information regarding the parental &/or progeny generation molecules; and 4) optionally repeating any of steps 1) to 3). In one aspect, there is generated (e.g., from a parent polynucleotide template), in what is termed "codon site-saturation mutagenesis," a progeny generation of polynucleotides, each having at least one set of up to three contiguous point mutations (i.e. different bases comprising a new codon), such that every codon (or every family of degenerate codons encoding the same amino acid) is represented at each codon position. Corresponding to, and encoded by, this progeny generation of polynucleotides, there is also generated a set of progeny polypeptides, each having at least one single amino acid point mutation. In a one aspect, there is generated, in what is termed "amino acid site-saturation mutagenesis", one such mutant polypeptide for each of the 19 naturally encoded polypeptide-forming alpha-amino acid substitutions at each and every amino acid position along the polypeptide. This yields, for each and every amino acid position along the parental polypeptide, a total of 20 distinct progeny polypeptides including the original amino acid, or potentially more than 21 distinct progeny polypeptides if additional amino acids are used either instead of or in addition to the 20 naturally encoded amino acids

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Thus, in another aspect, this approach is also serviceable for generating mutants containing, in addition to &/or in combination with the 20 naturally encoded polypeptide-forming alpha-amino acids, other rare &/or not naturally-encoded amino acids and amino acid derivatives. In yet another aspect, this approach is also serviceable for generating mutants by the use of, in addition to &/or in combination with natural or unaltered codon recognition systems of suitable hosts, altered, mutagenized, &/or designer codon recognition systems (such as in a host cell with one or more altered tRNA molecules.

In yet another aspect, this invention relates to recombination and more specifically to a method for preparing polynucleotides encoding a polypeptide by a method of *in vivo* re-assortment of polynucleotide sequences containing regions of partial homology, assembling the polynucleotides to form at least one polynucleotide and screening the polynucleotides for the production of polypeptide(s) having a useful property.

In yet another aspect, this invention is serviceable for analyzing and cataloguing, with respect to any molecular property (e.g. an enzymatic activity) or combination of properties allowed by current technology, the effects of any mutational change achieved (including particularly saturation mutagenesis). Thus, a comprehensive method is provided for determining the effect of changing each amino acid in a parental polypeptide into each of at least 19 possible substitutions. This allows each amino acid in a parental polypeptide to be characterized and catalogued according to its spectrum of potential effects on a measurable property of the polypeptide.

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In one aspect, an intron may be introduced into a chimeric progeny molecule by way of a nucleic acid building block. Introns often have consensus sequences at both termini in order to render them operational. In addition to enabling gene splicing, introns may serve an additional purpose by providing sites of homology to other nucleic acids to enable homologous recombination. For this purpose, and potentially others, it may be sometimes desirable to generate a large nucleic acid building block for introducing an intron. If the size is overly large easily generating by direct chemical synthesis of two single stranded oligos, such a specialized nucleic acid building block may also be generated by direct chemical synthesis of more than two single stranded oligos or by using a polymerase-based amplification reaction

The mutually compatible ligatable ends of the nucleic acid building blocks to be assembled are considered to be "serviceable" for this type of ordered assembly if they enable the building blocks to be coupled in predetermined orders. Thus, in one aspect, the overall assembly order in which the nucleic acid building blocks can be coupled is specified by the design of the ligatable ends and, if more than one assembly step is to be used, then the overall assembly order in which the nucleic acid building blocks can be coupled is also specified by the sequential order of the assembly step(s). In a one aspect of the invention, the annealed building pieces are treated with an enzyme, such as a ligase (e.g., T4 DNA ligase) to achieve covalent bonding of the building pieces.

Coupling can occur in a manner that does not make use of every nucleotide in a participating overhang. The coupling is particularly lively to survive (e.g. in a transformed host) if the coupling reinforced by treatment with a ligase enzyme to form what may be referred to as a "gap ligation" or a "gapped ligation". This type of coupling can contribute to generation of unwanted background product(s), but it can also be used advantageously increase the diversity of the progeny library generated by the designed ligation reassembly. Certain overhangs are able to undergo self-coupling to form a palindromic coupling. A

coupling is strengthened substantially if it is reinforced by treatment with a ligase enzyme. Lack of 5' phosphates on these overhangs can be used advantageously to prevent this type of palindromic self-ligation. Accordingly, this invention provides that nucleic acid building blocks can be chemically made (or ordered) that lack a 5' phosphate group. Alternatively, they can be removed, e.g. by treatment with a phosphatase enzyme, such as a calf intestinal alkaline phosphatase (CIAP), in order to prevent palindromic self-ligations in ligation reassembly processes.

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In a another aspect, the design of nucleic acid building blocks is obtained upon analysis of the sequences of a set of progenitor nucleic acid templates that serve as a basis for producing a progeny set of finalized chimeric nucleic acid molecules. These progenitor nucleic acid templates thus serve as a source of sequence information that aids in the design of the nucleic acid building blocks that are to be mutagenized, *i.e.* chimerized or shuffled.

In one exemplification, the invention provides for the chimerization of a family of related genes and their encoded family of related products. In a particular exemplification, the encoded products are enzymes. The xylanases of the present invention can be mutagenized in accordance with the methods described herein.

Thus according to one aspect of the invention, the sequences of a plurality of progenitor nucleic acid templates (e.g., polynucleotides of Group' A nucleic acid sequences) are aligned in order to select one or more demarcation points, which demarcation points can be located at an area of homology. The demarcation points can be used to delineate the boundaries of nucleic acid building blocks to be generated. Thus, the demarcation points identified and selected in the progenitor molecules serve as potential chimerization points in the assembly of the progeny molecules.

Typically a serviceable demarcation point is an area of homology (comprised of at least one homologous nucleotide base) shared by at least two progenitor templates, but the demarcation point can be an area of homology that is shared by at least half of the progenitor templates, at least two thirds of the progenitor templates, at least three fourths of the progenitor templates and preferably at almost all of the progenitor templates. Even more preferably still a serviceable demarcation point is an area of homology that is shared by all of the progenitor templates.

In a one aspect, the gene reassembly process is performed exhaustively in order to generate an exhaustive library. In other words, all possible ordered combinations of the nucleic acid building blocks are represented in the set of finalized chimeric nucleic acid molecules. At the same time, the assembly order (i.e. the order of assembly of each building

block in the 5' to 3 sequence of each finalized chimeric nucleic acid) in each combination is by design (or non-stochastic). Because of the non-stochastic nature of the method, the possibility of unwanted side products is greatly reduced.

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In another aspect, the method provides that the gene reassembly process is performed systematically, for example to generate a systematically compartmentalized library, with compartments that can be screened systematically, e.g., one by one. In other words the invention provides that, through the selective and judicious use of specific nucleic acid building blocks, coupled with the selective and judicious use of sequentially stepped assembly reactions, an experimental design can be achieved where specific sets of progeny products are made in each of several reaction vessels. This allows a systematic examination and screening procedure to be performed. Thus, it allows a potentially very large number of progeny molecules to be examined systematically in smaller groups.

Because of its ability to perform chimerizations in a manner that is highly flexible yet exhaustive and systematic as well, particularly when there is a low level of homology among the progenitor molecules, the instant invention provides for the generation of a library (or set) comprised of a large number of progeny molecules. Because of the non-stochastic nature of the instant gene reassembly invention, the progeny molecules generated preferably comprise a library of finalized chimeric nucleic acid molecules having an overall assembly order that is chosen by design. In a particularly aspect, such a generated library is comprised of greater than 10³ to greater than 10¹⁰⁰⁰ different progeny molecular species.

In one aspect, a set of finalized chimeric nucleic acid molecules, produced as described is comprised of a polynucleotide encoding a polypeptide. According to one aspect, this polynucleotide is a gene, which may be a man-made gene. According to another aspect, this polynucleotide is a gene pathway, which may be a man-made gene pathway. The invention provides that one or more man-made genes generated by the invention may be incorporated into a man-made gene pathway, such as pathway operable in a eukaryotic organism (including a plant).

In another exemplification, the synthetic nature of the step in which the building blocks are generated allows the design and introduction of nucleotides (e.g., one or more nucleotides, which may be, for example, codons or introns or regulatory sequences) that can later be optionally removed in an *in vitro* process (e.g., by mutagenesis) or in an *in vivo* process (e.g., by utilizing the gene splicing ability of a host organism). It is appreciated that in many instances the introduction of these nucleotides may also be desirable for many other reasons in addition to the potential benefit of creating a serviceable demarcation point.

Thus, according to another aspect, the invention provides that a nucleic acid building block can be used to introduce an intron. Thus, the invention provides that functional introns may be introduced into a man-made gene of the invention. The invention also provides that functional introns may be introduced into a man-made gene pathway of the invention. Accordingly, the invention provides for the generation of a chimeric polynucleotide that is a man-made gene containing one (or more) artificially introduced intron(s).

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Accordingly, the invention also provides for the generation of a chimeric polynucleotide that is a man-made gene pathway containing one (or more) artificially introduced intron(s). Preferably, the artificially introduced intron(s) are functional in one or more host cells for gene splicing much in the way that naturally-occurring introns serve functionally in gene splicing. The invention provides a process of producing man-made intron-containing polynucleotides to be introduced into host organisms for recombination and/or splicing.

A man-made gene produced using the invention can also serve as a substrate for recombination with another nucleic acid. Likewise, a man-made gene pathway produced using the invention can also serve as a substrate for recombination with another nucleic acid. In a one aspect, the recombination is facilitated by, or occurs at, areas of homology between the man-made, intron-containing gene and a nucleic acid, which serves as a recombination partner. In one aspect, the recombination partner may also be a nucleic acid generated by the invention, including a man-made gene or a man-made gene pathway. Recombination may be facilitated by or may occur at areas of homology that exist at the one (or more) artificially introduced intron(s) in the man-made gene.

The synthetic gene reassembly method of the invention utilizes a plurality of nucleic acid building blocks, each of which preferably has two ligatable ends. The two ligatable ends on each nucleic acid building block may be two blunt ends (*i.e.* each having an overhang of zero nucleotides), or preferably one blunt end and one overhang, or more preferably still two overhangs.

A useful overhang for this purpose may be a 3' overhang or a 5' overhang. Thus, a nucleic acid building block may have a 3' overhang or alternatively a 5' overhang or alternatively two 3' overhangs or alternatively two 5' overhangs. The overall order in which the nucleic acid building blocks are assembled to form a finalized chimeric nucleic acid molecule is determined by purposeful experimental design and is not random.

In one aspect, a nucleic acid building block is generated by chemical synthesis of two single-stranded nucleic acids (also referred to as single-stranded oligos) and contacting them so as to allow them to anneal to form a double-stranded nucleic acid building block.

A double-stranded nucleic acid building block can be of variable size. The sizes of these building blocks can be small or large. Exemplary sizes for building block range from 1 base pair (not including any overhangs) to 100,000 base pairs (not including any overhangs). Other exemplary size ranges are also provided, which have lower limits of from 1 bp to 10,000 bp (including every integer value in between) and upper limits of from 2 bp to 100,000 bp (including every integer value in between).

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Many methods exist by which a double-stranded nucleic acid building block can be generated that is serviceable for the invention; and these are known in the art and can be readily performed by the skilled artisan.

According to one aspect, a double-stranded nucleic acid building block is generated by first generating two single stranded nucleic acids and allowing them to anneal to form a double-stranded nucleic acid building block. The two strands of a double-stranded nucleic acid building block may be complementary at every nucleotide apart from any that form an overhang; thus containing no mismatches, apart from any overhang(s). According to another aspect, the two strands of a double-stranded nucleic acid building block are complementary at fewer than every nucleotide apart from any that form an overhang. Thus, according to this aspect, a double-stranded nucleic acid building block can be used to introduce codon degeneracy. Preferably the codon degeneracy is introduced using the site-saturation mutagenesis described herein, using one or more N,N,G/T cassettes or alternatively using one or more N,N,N cassettes.

The *in vivo* recombination method of the invention can be performed blindly on a pool of unknown hybrids or alleles of a specific polynucleotide or sequence. However, it is not necessary to know the actual DNA or RNA sequence of the specific polynucleotide.

The approach of using recombination within a mixed population of genes can be useful for the generation of any useful proteins, for example, interleukin I, antibodies, tPA and growth hormone. This approach may be used to generate proteins having altered specificity or activity. The approach may also be useful for the generation of hybrid nucleic acid sequences, for example, promoter regions, introns, exons, enhancer sequences, 31 untranslated regions or 51 untranslated regions of genes. Thus this approach may be used to generate genes having increased rates of expression. This approach may also be useful in the

study of repetitive DNA sequences. Finally, this approach may be useful to mutate ribozymes or aptamers.

In one aspect the invention described herein is directed to the use of repeated cycles of reductive reassortment, recombination and selection which allow for the directed molecular evolution of highly complex linear sequences, such as DNA, RNA or proteins thorough recombination.

Optimized Directed Evolution System

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The invention provides a non-stochastic gene modification system termed "optimized directed evolution system" to generate polypeptides, e.g., xylanases or antibodies of the invention, with new or altered properties. Optimized directed evolution is directed to the use of repeated cycles of reductive reassortment, recombination and selection that allow for the directed molecular evolution of nucleic acids through recombination. Optimized directed evolution allows generation of a large population of evolved chimeric sequences, wherein the generated population is significantly enriched for sequences that have a predetermined number of crossover events.

A crossover event is a point in a chimeric sequence where a shift in sequence occurs from one parental variant to another parental variant. Such a point is normally at the juncture of where oligonucleotides from two parents are ligated together to form a single sequence. This method allows calculation of the correct concentrations of oligonucleotide sequences so that the final chimeric population of sequences is enriched for the chosen number of crossover events. This provides more control over choosing chimeric variants having a predetermined number of crossover events.

In addition, this method provides a convenient means for exploring a tremendous amount of the possible protein variant space in comparison to other systems. Previously, if one generated, for example, 10^{13} chimeric molecules during a reaction, it would be extremely difficult to test such a high number of chimeric variants for a particular activity. Moreover, a significant portion of the progeny population would have a very high number of crossover events which resulted in proteins that were less likely to have increased levels of a particular activity. By using these methods, the population of chimerics molecules can be enriched for those variants that have a particular number of crossover events. Thus, although one can still generate 10^{13} chimeric molecules during a reaction, each of the molecules chosen for further analysis most likely has, for example, only three crossover events.

Because the resulting progeny population can be skewed to have a predetermined number of

crossover events, the boundaries on the functional variety between the chimeric molecules is reduced. This provides a more manageable number of variables when calculating which oligonucleotide from the original parental polynucleotides might be responsible for affecting a particular trait.

One method for creating a chimeric progeny polynucleotide sequence is to create oligonucleotides corresponding to fragments or portions of each parental sequence. Each oligonucleotide preferably includes a unique region of overlap so that mixing the oligonucleotides together results in a new variant that has each oligonucleotide fragment assembled in the correct order. Additional information can also be found, e.g., in USSN 09/332,835; U.S. Patent No. 6,361,974.

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The number of oligonucleotides generated for each parental variant bears a relationship to the total number of resulting crossovers in the chimeric molecule that is ultimately created. For example, three parental nucleotide sequence variants might be provided to undergo a ligation reaction in order to find a chimeric variant having, for example, greater activity at high temperature. As one example, a set of 50 oligonucleotide sequences can be generated corresponding to each portions of each parental variant.

Accordingly, during the ligation reassembly process there could be up to 50 crossover events within each of the chimeric sequences. The probability that each of the generated chimeric polynucleotides will contain oligonucleotides from each parental variant in alternating order is very low. If each oligonucleotide fragment is present in the ligation reaction in the same molar quantity it is likely that in some positions oligonucleotides from the same parental polynucleotide will ligate next to one another and thus not result in a crossover event. If the concentration of each oligonucleotide from each parent is kept constant during any ligation step in this example, there is a 1/3 chance (assuming 3 parents) that an oligonucleotide from the same parental variant will ligate within the chimeric sequence and produce no crossover.

Accordingly, a probability density function (PDF) can be determined to predict the population of crossover events that are likely to occur during each step in a ligation reaction given a set number of parental variants, a number of oligonucleotides corresponding to each variant, and the concentrations of each variant during each step in the ligation reaction. The statistics and mathematics behind determining the PDF is described below. By utilizing these methods, one can calculate such a probability density function, and thus enrich the chimeric progeny population for a predetermined number of crossover events resulting from a particular ligation reaction. Moreover, a target number of crossover events can be predetermined, and the system then programmed to calculate the starting quantities of

each parental oligonucleotide during each step in the ligation reaction to result in a probability density function that centers on the predetermined number of crossover events. These methods are directed to the use of repeated cycles of reductive reassortment, recombination and selection that allow for the directed molecular evolution of a nucleic acid encoding a polypeptide through recombination. This system allows generation of a large population of evolved chimeric sequences, wherein the generated population is significantly enriched for sequences that have a predetermined number of crossover events. A crossover event is a point in a chimeric sequence where a shift in sequence occurs from one parental variant to another parental variant. Such a point is normally at the juncture of where oligonucleotides from two parents are ligated together to form a single sequence. The method allows calculation of the correct concentrations of oligonucleotide sequences so that the final chimeric population of sequences is enriched for the chosen number of crossover events. This provides more control over choosing chimeric variants having a predetermined number of crossover events.

In addition, these methods provide a convenient means for exploring a tremendous amount of the possible protein variant space in comparison to other systems. By using the methods described herein, the population of chimerics molecules can be enriched for those variants that have a particular number of crossover events. Thus, although one can still generate 10¹³ chimeric molecules during a reaction, each of the molecules chosen for further analysis most likely has, for example, only three crossover events. Because the resulting progeny population can be skewed to have a predetermined number of crossover events, the boundaries on the functional variety between the chimeric molecules is reduced. This provides a more manageable number of variables when calculating which oligonucleotide from the original parental polynucleotides might be responsible for affecting a particular trait.

In one aspect, the method creates a chimeric progeny polynucleotide sequence by creating oligonucleotides corresponding to fragments or portions of each parental sequence. Each oligonucleotide preferably includes a unique region of overlap so that mixing the oligonucleotides together results in a new variant that has each oligonucleotide fragment assembled in the correct order. See also USSN 09/332,835.

Determining Crossover Events

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Aspects of the invention include a system and software that receive a desired crossover probability density function (PDF), the number of parent genes to be reassembled,

and the number of fragments in the reassembly as inputs. The output of this program is a "fragment PDF" that can be used to determine a recipe for producing reassembled genes, and the estimated crossover PDF of those genes. The processing described herein is preferably performed in MATLABTM (The Mathworks, Natick, Massachusetts) a programming language and development environment for technical computing.

Iterative Processes

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In practicing the invention, these processes can be iteratively repeated. For example, a nucleic acid (or, the nucleic acid) responsible for an altered or new xylanase phenotype is identified, re-isolated, again modified, re-tested for activity. This process can be iteratively repeated until a desired phenotype is engineered. For example, an entire biochemical anabolic or catabolic pathway can be engineered into a cell, including, e.g., xylanase activity.

Similarly, if it is determined that a particular oligonucleotide has no affect at all on the desired trait (e.g., a new xylanase phenotype), it can be removed as a variable by synthesizing larger parental oligonucleotides that include the sequence to be removed. Since incorporating the sequence within a larger sequence prevents any crossover events, there will no longer be any variation of this sequence in the progeny polynucleotides. This iterative practice of determining which oligonucleotides are most related to the desired trait, and which are unrelated, allows more efficient exploration all of the possible protein variants that might be provide a particular trait or activity.

In vivo shuffling

In vivo shuffling of molecules is use in methods of the invention that provide variants of polypeptides of the invention, e.g., antibodies, xylanases, and the like. In vivo shuffling can be performed utilizing the natural property of cells to recombine multimers. While recombination in vivo has provided the major natural route to molecular diversity, genetic recombination remains a relatively complex process that involves 1) the recognition of homologies; 2) strand cleavage, strand invasion, and metabolic steps leading to the production of recombinant chiasma; and finally 3) the resolution of chiasma into discrete recombined molecules. The formation of the chiasma requires the recognition of homologous sequences.

In another aspect, the invention includes a method for producing a hybrid polynucleotide from at least a first polynucleotide and a second polynucleotide. The invention can be used to produce a hybrid polynucleotide by introducing at least a first

polynucleotide and a second polynucleotide which share at least one region of partial sequence homology (e.g., SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257 and combinations thereof) into a suitable host cell. The regions of partial sequence homology promote processes which result in sequence reorganization producing a hybrid polynucleotide. The 10 term "hybrid polynucleotide", as used herein, is any nucleotide sequence which results from the method of the present invention and contains sequence from at least two original polynucleotide sequences. Such hybrid polynucleotides can result from intermolecular recombination events which promote sequence integration between DNA molecules. In addition, such hybrid polynucleotides can result from intramolecular reductive reassortment 15 processes which utilize repeated sequences to alter a nucleotide sequence within a DNA molecule.

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In vivo reassortment is focused on "inter-molecular" processes collectively referred to as "recombination" which in bacteria, is generally viewed as a "RecA-dependent" phenomenon. The invention can rely on recombination processes of a host cell to recombine and re-assort sequences, or the cells' ability to mediate reductive processes to decrease the complexity of quasi-repeated sequences in the cell by deletion. This process of "reductive reassortment" occurs by an "intra-molecular", RecA-independent process.

Therefore, in another aspect of the invention, novel polynucleotides can be generated by the process of reductive reassortment. The method involves the generation of constructs containing consecutive sequences (original encoding sequences), their insertion into an appropriate vector and their subsequent introduction into an appropriate host cell. The reassortment of the individual molecular identities occurs by combinatorial processes between the consecutive sequences in the construct possessing regions of homology, or between quasi-repeated units. The reassortment process recombines and/or reduces the complexity and extent of the repeated sequences and results in the production of novel molecular species. Various treatments may be applied to enhance the rate of reassortment. These could include treatment with ultra-violet light, or DNA damaging chemicals and/or the use of host cell lines displaying enhanced levels of "genetic instability". Thus the

reassortment process may involve homologous recombination or the natural property of quasi-repeated sequences to direct their own evolution.

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Repeated or "quasi-repeated" sequences play a role in genetic instability. In the present invention, "quasi-repeats" are repeats that are not restricted to their original unit structure. Quasi-repeated units can be presented as an array of sequences in a construct; consecutive units of similar sequences. Once ligated, the junctions between the consecutive sequences become essentially invisible and the quasi-repetitive nature of the resulting construct is now continuous at the molecular level. The deletion process the cell performs to reduce the complexity of the resulting construct operates between the quasi-repeated sequences. The quasi-repeated units provide a practically limitless repertoire of templates upon which slippage events can occur. The constructs containing the quasi-repeats thus effectively provide sufficient molecular elasticity that deletion (and potentially insertion) events can occur virtually anywhere within the quasi-repetitive units.

When the quasi-repeated sequences are all ligated in the same orientation, for instance head to tail or vice versa, the cell cannot distinguish individual units. Consequently, the reductive process can occur throughout the sequences. In contrast, when for example, the units are presented head to head, rather than head to tail, the inversion delineates the endpoints of the adjacent unit so that deletion formation will favor the loss of discrete units. Thus, it is preferable with the present method that the sequences are in the same orientation.

Random orientation of quasi-repeated sequences will result in the loss of reassortment efficiency, while consistent orientation of the sequences will offer the highest efficiency. However, while having fewer of the contiguous sequences in the same orientation decreases the efficiency, it may still provide sufficient elasticity for the effective recovery of novel molecules. Constructs can be made with the quasi-repeated sequences in the same orientation to allow higher efficiency.

Sequences can be assembled in a head to tail orientation using any of a variety of methods, including the following:

- a) Primers that include a poly-A head and poly-T tail which when made singlestranded would provide orientation can be utilized. This is accomplished by having the first few bases of the primers made from RNA and hence easily removed RNAseH.
- b) Primers that include unique restriction cleavage sites can be utilized. Multiple sites, a battery of unique sequences and repeated synthesis and ligation steps would be required.

c) The inner few bases of the primer could be thiolated and an exonuclease used to produce properly tailed molecules.

The recovery of the re-assorted sequences relies on the identification of cloning vectors with a reduced repetitive index (RI). The re-assorted encoding sequences can then be recovered by amplification. The products are re-cloned and expressed. The recovery of cloning vectors with reduced RI can be affected by:

1) The use of vectors only stably maintained when the construct is reduced in complexity.

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- The physical recovery of shortened vectors by physical procedures. In this case, the cloning vector would be recovered using standard plasmid isolation procedures and size fractionated on either an agarose gel, or column with a low molecular weight cut off utilizing standard procedures.
 - 3) The recovery of vectors containing interrupted genes which can be selected when insert size decreases.
- The use of direct selection techniques with an expression vector and the appropriate selection.

Encoding sequences (for example, genes) from related organisms may demonstrate a high degree of homology and encode quite diverse protein products. These types of sequences are particularly useful in the present invention as quasi-repeats. However, while the examples illustrated below demonstrate the reassortment of nearly identical original encoding sequences (quasi-repeats), this process is not limited to such nearly identical repeats.

The following example demonstrates a method of the invention. Encoding nucleic acid sequences (quasi-repeats) derived from three (3) unique species are described. Each sequence encodes a protein with a distinct set of properties. Each of the sequences differs by a single or a few base pairs at a unique position in the sequence. The quasi-repeated sequences are separately or collectively amplified and ligated into random assemblies such that all possible permutations and combinations are available in the population of ligated molecules. The number of quasi-repeat units can be controlled by the assembly conditions. The average number of quasi-repeated units in a construct is defined as the repetitive index (RI).

Once formed, the constructs may, or may not be size fractionated on an agarose gel according to published protocols, inserted into a cloning vector and transfected

into an appropriate host cell. The cells are then propagated and "reductive reassortment" is effected. The rate of the reductive reassortment process may be stimulated by the introduction of DNA damage if desired. Whether the reduction in RI is mediated by deletion formation between repeated sequences by an "intra-molecular" mechanism, or mediated by recombination-like events through "inter-molecular" mechanisms is immaterial. The end result is a reassortment of the molecules into all possible combinations.

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Optionally, the method comprises the additional step of screening the library members of the shuffled pool to identify individual shuffled library members having the ability to bind or otherwise interact, or catalyze a particular reaction (e.g., such as catalytic domain of an enzyme) with a predetermined macromolecule, such as for example a proteinaceous receptor, an oligosaccharide, virion, or other predetermined compound or structure.

The polypeptides that are identified from such libraries can be used for therapeutic, diagnostic, research and related purposes (e.g., catalysts, solutes for increasing osmolarity of an aqueous solution and the like) and/or can be subjected to one or more additional cycles of shuffling and/or selection.

In another aspect, it is envisioned that prior to or during recombination or reassortment, polynucleotides generated by the method of the invention can be subjected to agents or processes which promote the introduction of mutations into the original polynucleotides. The introduction of such mutations would increase the diversity of resulting hybrid polynucleotides and polypeptides encoded therefrom. The agents or processes which promote mutagenesis can include, but are not limited to: (+)-CC-1065, or a synthetic analog such as (+)-CC-1065-(N3-Adenine (See Sun and Hurley, (1992); an N-acetylated or deacetylated 4'-fluro-4-aminobiphenyl adduct capable of inhibiting DNA synthesis (See, for example, van de Poll et al. (1992)); or a N-acetylated or deacetylated 4-aminobiphenyl adduct capable of inhibiting DNA synthesis (See also, van de Poll et al. (1992), pp. 751-758); trivalent chromium, a trivalent chromium salt, a polycyclic aromatic hydrocarbon (PAH) DNA adduct capable of inhibiting DNA replication, such as 7-bromomethylbenz[a]anthracene ("BMA"), tris(2,3-dibromopropyl)phosphate ("Tris-BP"), 1,2-dibromo-3chloropropane ("DBCP"), 2-bromoacrolein (2BA), benzo[a]pyrene-7,8-dihydrodiol-9-10epoxide ("BPDE"), a platinum(II) halogen salt, N-hydroxy-2-amino-3-methylimidazo[4,5-f]quinoline ("N-hydroxy-IQ") and N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-f]pyridine ("N-hydroxy-PhIP"). Exemplary means for slowing or halting PCR amplification consist of UV light (+)-CC-1065 and (+)-CC-1065-(N3-Adenine). Particularly encompassed

means are DNA adducts or polynucleotides comprising the DNA adducts from the polynucleotides or polynucleotides pool, which can be released or removed by a process including heating the solution comprising the polynucleotides prior to further processing.

In another aspect the invention is directed to a method of producing recombinant proteins having biological activity by treating a sample comprising doublestranded template polynucleotides encoding a wild-type protein under conditions according to the invention which provide for the production of hybrid or re-assorted polynucleotides.

Producing sequence variants

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The invention also provides additional methods for making sequence variants of the nucleic acid (e.g., xylanase) sequences of the invention. The invention also provides additional methods for isolating xylanases using the nucleic acids and polypeptides of the invention. In one aspect, the invention provides for variants of a xylanase coding sequence (e.g., a gene, cDNA or message) of the invention, which can be altered by any means, including, e.g., random or stochastic methods, or, non-stochastic, or "directed evolution," 15 methods, as described above.

The isolated variants may be naturally occurring. Variant can also be created in vitro. Variants may be created using genetic engineering techniques such as site directed mutagenesis, random chemical mutagenesis, Exonuclease III deletion procedures, and standard cloning techniques. Alternatively, such variants, fragments, analogs, or derivatives may be created using chemical synthesis or modification procedures. Other methods of making variants are also familiar to those skilled in the art. These include procedures in which nucleic acid sequences obtained from natural isolates are modified to generate nucleic acids which encode polypeptides having characteristics which enhance their value in industrial or laboratory applications. In such procedures, a large number of variant sequences having one or more nucleotide differences with respect to the sequence obtained from the natural isolate are generated and characterized. These nucleotide differences can result in amino acid changes with respect to the polypeptides encoded by the nucleic acids from the natural isolates.

For example, variants may be created using error prone PCR. In error prone PCR, PCR is performed under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product. Error prone PCR is described, e.g., in Leung, D.W., et al., Technique, 1:11-15, 1989) and Caldwell, R. C. & Joyce G.F., PCR Methods Applic., 2:28-33, 1992. Briefly, in

such procedures, nucleic acids to be mutagenized are mixed with PCR primers, reaction buffer, MgCl₂, MnCl₂, Taq polymerase and an appropriate concentration of dNTPs for achieving a high rate of point mutation along the entire length of the PCR product. For example, the reaction may be performed using 20 fmoles of nucleic acid to be mutagenized, 30 pmole of each PCR primer, a reaction buffer comprising 50mM KCl, 10mM Tris HCl (pH 8.3) and 0.01% gelatin, 7mM MgCl₂, 0.5mM MnCl₂, 5 units of Taq polymerase, 0.2mM dGTP, 0.2mM dATP, 1mM dCTP, and 1mM dTTP. PCR may be performed for 30 cycles of 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min. However, it will be appreciated that these parameters may be varied as appropriate. The mutagenized nucleic acids are cloned into an appropriate vector and the activities of the polypeptides encoded by the mutagenized nucleic acids are evaluated.

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Variants may also be created using oligonucleotide directed mutagenesis to generate site-specific mutations in any cloned DNA of interest. Oligonucleotide mutagenesis is described, e.g., in Reidhaar-Olson (1988) Science 241:53-57. Briefly, in such procedures a plurality of double stranded oligonucleotides bearing one or more mutations to be introduced into the cloned DNA are synthesized and inserted into the cloned DNA to be mutagenized. Clones containing the mutagenized DNA are recovered and the activities of the polypeptides they encode are assessed.

Another method for generating variants is assembly PCR. Assembly PCR involves the assembly of a PCR product from a mixture of small DNA fragments. A large number of different PCR reactions occur in parallel in the same vial, with the products of one reaction priming the products of another reaction. Assembly PCR is described in, e.g., U.S. Patent No. 5,965,408.

Still another method of generating variants is sexual PCR mutagenesis. In sexual PCR mutagenesis, forced homologous recombination occurs between DNA molecules of different but highly related DNA sequence *in vitro*, as a result of random fragmentation of the DNA molecule based on sequence homology, followed by fixation of the crossover by primer extension in a PCR reaction. Sexual PCR mutagenesis is described, e.g., in Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751. Briefly, in such procedures a plurality of nucleic acids to be recombined are digested with DNase to generate fragments having an average size of 50-200 nucleotides. Fragments of the desired average size are purified and resuspended in a PCR mixture. PCR is conducted under conditions which facilitate recombination between the nucleic acid fragments. For example, PCR may be performed by resuspending the purified fragments at a concentration of 10-30ng/µl in a solution of 0.2mM

of each dNTP, 2.2mM MgCl₂, 50mM KCL, 10mM Tris HCl, pH 9.0, and 0.1% Triton X-100. 2.5 units of Taq polymerase per 100:1 of reaction mixture is added and PCR is performed using the following regime: 94°C for 60 seconds, 94°C for 30 seconds, 50-55°C for 30 seconds, 72°C for 30 seconds (30-45 times) and 72°C for 5 minutes. However, it will be appreciated that these parameters may be varied as appropriate. In some aspects, oligonucleotides may be included in the PCR reactions. In other aspects, the Klenow fragment of DNA polymerase I may be used in a first set of PCR reactions and Taq polymerase may be used in a subsequent set of PCR reactions. Recombinant sequences are isolated and the activities of the polypeptides they encode are assessed.

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Variants may also be created by *in vivo* mutagenesis. In some aspects, random mutations in a sequence of interest are generated by propagating the sequence of interest in a bacterial strain, such as an E. coli strain, which carries mutations in one or more of the DNA repair pathways. Such "mutator" strains have a higher random mutation rate than that of a wild-type parent. Propagating the DNA in one of these strains will eventually generate random mutations within the DNA. Mutator strains suitable for use for *in vivo* mutagenesis are described in PCT Publication No. WO 91/16427, published October 31, 1991, entitled "Methods for Phenotype Creation from Multiple Gene Populations".

Variants may also be generated using cassette mutagenesis. In cassette mutagenesis a small region of a double stranded DNA molecule is replaced with a synthetic oligonucleotide "cassette" that differs from the native sequence. The oligonucleotide often contains completely and/or partially randomized native sequence.

Recursive ensemble mutagenesis may also be used to generate variants. Recursive ensemble mutagenesis is an algorithm for protein engineering (protein mutagenesis) developed to produce diverse populations of phenotypically related mutants whose members differ in amino acid sequence. This method uses a feedback mechanism to control successive rounds of combinatorial cassette mutagenesis. Recursive ensemble mutagenesis is described in Arkin, A.P. and Youvan, D.C., PNAS, USA, 89:7811-7815, 1992.

In some aspects, variants are created using exponential ensemble mutagenesis.

Exponential ensemble mutagenesis is a process for generating combinatorial libraries with a high percentage of unique and functional mutants, wherein small groups of residues are randomized in parallel to identify, at each altered position, amino acids which lead to functional proteins. Exponential ensemble mutagenesis is described in Delegrave, S. and Youvan, D.C., Biotechnology Research, 11:1548-1552, 1993. Random and site-directed

mutagenesis are described in Arnold, F.H., Current Opinion in Biotechnology, 4:450-455, 1993.

In some aspects, the variants are created using shuffling procedures wherein portions of a plurality of nucleic acids which encode distinct polypeptides are fused together to create chimeric nucleic acid sequences which encode chimeric polypeptides as described in U.S. Patent No. 5,965,408, filed July 9, 1996, entitled, "Method of DNA Reassembly by Interrupting Synthesis" and U.S. Patent No. 5,939,250, filed May 22, 1996, entitled, "Production of Enzymes Having Desired Activities by Mutagenesis.

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The variants of the polypeptides of Group B amino acid sequences may be variants in which one or more of the amino acid residues of the polypeptides of the Group B amino acid sequences are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code.

Conservative substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the following replacements: replacements of an aliphatic amino acid such as Alanine, Valine, Leucine and Isoleucine with another aliphatic amino acid; replacement of a Serine with a Threonine or vice versa; replacement of an acidic residue such as Aspartic acid and Glutamic acid with another acidic residue; replacement of a residue bearing an amide group, such as Asparagine and Glutamine, with another residue bearing an amide group; exchange of a basic residue such as Lysine and Arginine with another basic residue; and replacement of an aromatic residue such as Phenylalanine, Tyrosine with another aromatic residue.

Other variants are those in which one or more of the amino acid residues of the polypeptides of the Group B amino acid sequences includes a substituent group.

Still other variants are those in which the polypeptide is associated with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol).

Additional variants are those in which additional amino acids are fused to the polypeptide, such as a leader sequence, a secretory sequence, a proprotein sequence or a sequence which facilitates purification, enrichment, or stabilization of the polypeptide.

In some aspects, the fragments, derivatives and analogs retain the same biological function or activity as the polypeptides of Group B amino acid sequences and sequences substantially identical thereto. In other aspects, the fragment, derivative, or analog

includes a proprotein, such that the fragment, derivative, or analog can be activated by cleavage of the proprotein portion to produce an active polypeptide.

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Optimizing codons to achieve high levels of protein expression in host cells

The invention provides methods for modifying xylanase-encoding nucleic acids to modify codon usage. In one aspect, the invention provides methods for modifying codons in a nucleic acid encoding a xylanase to increase or decrease its expression in a host cell. The invention also provides nucleic acids encoding a xylanase modified to increase its expression in a host cell, xylanase so modified, and methods of making the modified xylanases. The method comprises identifying a "non-preferred" or a "less preferred" codon in xylanase-encoding nucleic acid and replacing one or more of these non-preferred or less preferred codons with a "preferred codon" encoding the same amino acid as the replaced codon and at least one non-preferred or less preferred codon in the nucleic acid has been replaced by a preferred codon encoding the same amino acid. A preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell.

Host cells for expressing the nucleic acids, expression cassettes and vectors of the invention include bacteria, yeast, fungi, plant cells, insect cells and mammalian cells. Thus, the invention provides methods for optimizing codon usage in all of these cells, codonaltered nucleic acids and polypeptides made by the codon-altered nucleic acids. Exemplary host cells include gram negative bacteria, such as Escherichia coli and Pseudomonas fluorescens; gram positive bacteria, such as Streptomyces diversa, Lactobacillus gasseri, Lactococcus lactis, Lactococcus cremoris, Bacillus subtilis. Exemplary host cells also include eukaryotic organisms, e.g., various yeast, such as Saccharomyces sp., including Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, and Kluyveromyces lactis, Hansenula polymorpha, Aspergillus niger, and mammalian cells and cell lines and insect cells and cell lines. Thus, the invention also includes nucleic acids and polypeptides optimized for expression in these organisms and species.

For example, the codons of a nucleic acid encoding a xylanase isolated from a bacterial cell are modified such that the nucleic acid is optimally expressed in a bacterial cell different from the bacteria from which the xylanase was derived, a yeast, a fungi, a plant cell, an insect cell or a mammalian cell. Methods for optimizing codons are well known in the art, see, e.g., U.S. Patent No. 5,795,737; Baca (2000) Int. J. Parasitol. 30:113-118; Hale (1998) Protein Expr. Purif. 12:185-188; Narum (2001) Infect. Immun. 69:7250-7253. See also

Narum (2001) Infect. Immun. 69:7250-7253, describing optimizing codons in mouse systems; Outchkourov (2002) Protein Expr. Purif. 24:18-24, describing optimizing codons in yeast; Feng (2000) Biochemistry 39:15399-15409, describing optimizing codons in *E. coli*; Humphreys (2000) Protein Expr. Purif. 20:252-264, describing optimizing codon usage that affects secretion in *E. coli*.

Transgenic non-human animals

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The invention provides transgenic non-human animals comprising a nucleic acid, a polypeptide (e.g., a xylanase), an expression cassette or vector or a transfected or transformed cell of the invention. The invention also provides methods of making and using these transgenic non-human animals.

The transgenic non-human animals can be, e.g., goats, rabbits, sheep, pigs, cows, rats and mice, comprising the nucleic acids of the invention. These animals can be used, e.g., as in vivo models to study xylanase activity, or, as models to screen for agents that change the xylanase activity in vivo. The coding sequences for the polypeptides to be expressed in the transgenic non-human animals can be designed to be constitutive, or, under the control of tissue-specific, developmental-specific or inducible transcriptional regulatory factors. Transgenic non-human animals can be designed and generated using any method known in the art; see, e.g., U.S. Patent Nos. 6,211,428; 6,187,992; 6,156,952; 6,118,044; 6,111,166; 6,107,541; 5,959,171; 5,922,854; 5,892,070; 5,880,327; 5,891,698; 5,639,940; 5,573,933; 5,387,742; 5,087,571, describing making and using transformed cells and eggs and transgenic mice, rats, rabbits, sheep, pigs and cows. See also, e.g., Pollock (1999) J. Immunol. Methods 231:147-157, describing the production of recombinant proteins in the milk of transgenic dairy animals; Baguisi (1999) Nat. Biotechnol. 17:456-461, demonstrating the production of transgenic goats. U.S. Patent No. 6,211,428, describes making and using transgenic non-human mammals which express in their brains a nucleic acid construct comprising a DNA sequence. U.S. Patent No. 5,387,742, describes injecting cloned recombinant or synthetic DNA sequences into fertilized mouse eggs, implanting the injected eggs in pseudo-pregnant females, and growing to term transgenic mice whose cells express proteins related to the pathology of Alzheimer's disease. U.S. Patent No. 6,187,992, describes making and using a transgenic mouse whose genome comprises a disruption of the gene encoding amyloid precursor protein (APP).

"Knockout animals" can also be used to practice the methods of the invention. For example, in one aspect, the transgenic or modified animals of the invention comprise a "knockout animal," e.g., a "knockout mouse," engineered not to express an endogenous gene,

which is replaced with a gene expressing a xylanase of the invention, or, a fusion protein comprising a xylanase of the invention.

Transgenic Plants and Seeds

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The invention provides transgenic plants and seeds comprising a nucleic acid, a polypeptide (e.g., a xylanase), an expression cassette or vector or a transfected or transformed cell of the invention. The invention also provides plant products, e.g., oils, seeds, leaves, extracts and the like, comprising a nucleic acid and/or a polypeptide (e.g., a xylanase) of the invention. The transgenic plant can be dicotyledonous (a dicot) or monocotyledonous (a monocot). The invention also provides methods of making and using these transgenic plants and seeds. The transgenic plant or plant cell expressing a polypeptide of the present invention may be constructed in accordance with any method known in the art. See, for example, U.S. Patent No. 6,309,872.

Nucleic acids and expression constructs of the invention can be introduced into a plant cell by any means. For example, nucleic acids or expression constructs can be introduced into the genome of a desired plant host, or, the nucleic acids or expression constructs can be episomes. Introduction into the genome of a desired plant can be such that the host's xylanase production is regulated by endogenous transcriptional or translational control elements. The invention also provides "knockout plants" where insertion of gene sequence by, e.g., homologous recombination, has disrupted the expression of the endogenous gene. Means to generate "knockout" plants are well-known in the art, see, e.g., Strepp (1998) Proc Natl. Acad. Sci. USA 95:4368-4373; Miao (1995) Plant J 7:359-365. See discussion on transgenic plants, below.

The nucleic acids of the invention can be used to confer desired traits on essentially any plant, e.g., on starch-producing plants, such as potato, wheat, rice, barley, and the like. Nucleic acids of the invention can be used to manipulate metabolic pathways of a plant in order to optimize or alter host's expression of xylanase. The can change xylanase activity in a plant. Alternatively, a xylanase of the invention can be used in production of a transgenic plant to produce a compound not naturally produced by that plant. This can lower production costs or create a novel product.

In one aspect, the first step in production of a transgenic plant involves making an expression construct for expression in a plant cell. These techniques are well known in the art. They can include selecting and cloning a promoter, a coding sequence for facilitating efficient binding of ribosomes to mRNA and selecting the appropriate gene terminator sequences. One exemplary constitutive promoter is CaMV35S, from the cauliflower mosaic

virus, which generally results in a high degree of expression in plants. Other promoters are more specific and respond to cues in the plant's internal or external environment. An exemplary light-inducible promoter is the promoter from the cab gene, encoding the major chlorophyll a/b binding protein.

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In one aspect, the nucleic acid is modified to achieve greater expression in a plant cell. For example, a sequence of the invention is likely to have a higher percentage of A-T nucleotide pairs compared to that seen in a plant, some of which prefer G-C nucleotide pairs. Therefore, A-T nucleotides in the coding sequence can be substituted with G-C nucleotides without significantly changing the amino acid sequence to enhance production of the gene product in plant cells.

Selectable marker gene can be added to the gene construct in order to identify plant cells or tissues that have successfully integrated the transgene. This may be necessary because achieving incorporation and expression of genes in plant cells is a rare event, occurring in just a few percent of the targeted tissues or cells. Selectable marker genes encode proteins that provide resistance to agents that are normally toxic to plants, such as antibiotics or herbicides. Only plant cells that have integrated the selectable marker gene will survive when grown on a medium containing the appropriate antibiotic or herbicide. As for other inserted genes, marker genes also require promoter and termination sequences for proper function.

In one aspect, making transgenic plants or seeds comprises incorporating sequences of the invention and, optionally, marker genes into a target expression construct (e.g., a plasmid), along with positioning of the promoter and the terminator sequences. This can involve transferring the modified gene into the plant through a suitable method. For example, a construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation and microinjection of plant cell protoplasts, or the constructs can be introduced directly to plant tissue using ballistic methods, such as DNA particle bombardment. For example, see, e.g., Christou (1997) Plant Mol. Biol. 35:197-203; Pawlowski (1996) Mol. Biotechnol. 6:17-30; Klein (1987) Nature 327:70-73; Takumi (1997) Genes Genet. Syst. 72:63-69, discussing use of particle bombardment to introduce transgenes into wheat; and Adam (1997) supra, for use of particle bombardment to introduce YACs into plant cells. For example, Rinehart (1997) supra, used particle bombardment to generate transgenic cotton plants. Apparatus for accelerating particles is described U.S. Pat. No. 5,015,580; and, the commercially available BioRad (Biolistics) PDS-2000 particle

acceleration instrument; see also, John, U.S. Patent No. 5,608,148; and Ellis, U.S. Patent No. 5,681,730, describing particle-mediated transformation of gymnosperms.

In one aspect, protoplasts can be immobilized and injected with a nucleic acids, e.g., an expression construct. Although plant regeneration from protoplasts is not easy with cereals, plant regeneration is possible in legumes using somatic embryogenesis from protoplast derived callus. Organized tissues can be transformed with naked DNA using gene gun technique, where DNA is coated on tungsten microprojectiles, shot 1/100th the size of cells, which carry the DNA deep into cells and organelles. Transformed tissue is then induced to regenerate, usually by somatic embryogenesis. This technique has been successful in several cereal species including maize and rice.

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Nucleic acids, e.g., expression constructs, can also be introduced in to plant cells using recombinant viruses. Plant cells can be transformed using viral vectors, such as, e.g., tobacco mosaic virus derived vectors (Rouwendal (1997) Plant Mol. Biol. 33:989-999), see Porta (1996) "Use of viral replicons for the expression of genes in plants," Mol. Biotechnol. 5:209-221.

Alternatively, nucleic acids, e.g., an expression construct, can be combined with suitable T-DNA flanking regions and introduced into a conventional Agrobacterium tumefaciens host vector. The virulence functions of the Agrobacterium tumefaciens host will direct the insertion of the construct and adjacent marker into the plant cell DNA when the cell is infected by the bacteria. Agrobacterium tumefaciens-mediated transformation techniques, including disarming and use of binary vectors, are well described in the scientific literature. See, e.g., Horsch (1984) Science 233:496-498; Fraley (1983) Proc. Natl. Acad. Sci. USA 80:4803 (1983); Gene Transfer to Plants, Potrykus, ed. (Springer-Verlag, Berlin 1995). The DNA in an A. tumefaciens cell is contained in the bacterial chromosome as well as in another structure known as a Ti (tumor-inducing) plasmid. The Ti plasmid contains a stretch of DNA termed T-DNA (~20 kb long) that is transferred to the plant cell in the infection process and a series of vir (virulence) genes that direct the infection process. A. tumefaciens can only infect a plant through wounds: when a plant root or stem is wounded it gives off certain chemical signals, in response to which, the vir genes of A. tumefaciens become activated and direct a series of events necessary for the transfer of the T-DNA from the Ti plasmid to the plant's chromosome. The T-DNA then enters the plant cell through the wound. One speculation is that the T-DNA waits until the plant DNA is being replicated or transcribed, then inserts itself into the exposed plant DNA. In order to use A. tumefaciens as a transgene vector, the tumorinducing section of T-DNA have to be removed, while retaining the T-DNA border regions

and the vir genes. The transgene is then inserted between the T-DNA border regions, where it is transferred to the plant cell and becomes integrated into the plant's chromosomes.

The invention provides for the transformation of monocotyledonous plants using the nucleic acids of the invention, including important cereals, see Hiei (1997) Plant Mol. Biol. 35:205-218. See also, e.g., Horsch, Science (1984) 233:496; Fraley (1983) Proc. Natl. Acad. Sci USA 80:4803; Thykjaer (1997) supra; Park (1996) Plant Mol. Biol. 32:1135-1148, discussing T-DNA integration into genomic DNA. See also D'Halluin, U.S. Patent No. 5,712,135, describing a process for the stable integration of a DNA comprising a gene that is functional in a cell of a cereal, or other monocotyledonous plant.

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In one aspect, the third step can involve selection and regeneration of whole plants capable of transmitting the incorporated target gene to the next generation. Such regeneration techniques rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker that has been introduced together with the desired nucleotide sequences. Plant regeneration from cultured protoplasts is described in Evans et al., *Protoplasts Isolation and Culture*, *Handbook of Plant Cell Culture*, pp. 124-176, MacMillilan Publishing Company, New York, 1983; and Binding, *Regeneration of Plants*, *Plant Protoplasts*, pp. 21-73, CRC Press, Boca Raton, 1985.

Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee (1987) Ann. Rev. of Plant Phys. 38:467-486. To obtain whole plants from transgenic tissues such as immature embryos, they can be grown under controlled environmental conditions in a series of media containing nutrients and hormones, a process known as tissue culture. Once whole plants are generated and produce seed, evaluation of the progeny begins.

After the expression cassette is stably incorporated in transgenic plants, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed. Since transgenic expression of the nucleic acids of the invention leads to phenotypic changes, plants comprising the recombinant nucleic acids of the invention can be sexually crossed with a second plant to obtain a final product. Thus, the seed of the invention can be derived from a cross between two transgenic plants of the invention, or a cross between a plant of the invention and another plant. The desired effects (e.g., expression of the polypeptides of the invention to produce a plant in which flowering behavior is altered) can be enhanced when both parental plants express the polypeptides (e.g., a xylanase) of the invention. The desired effects can be passed to future plant generations by standard propagation means.

The nucleic acids and polypeptides of the invention are expressed in or inserted in any plant or seed. Transgenic plants of the invention can be dicotyledonous or monocotyledonous. Examples of monocot transgenic plants of the invention are grasses, such as meadow grass (blue grass, Poa), forage grass such as festuca, lolium, temperate grass, such as Agrostis, and cereals, e.g., wheat, oats, rye, barley, rice, sorghum, and maize (corn). Examples of dicot transgenic plants of the invention are tobacco, legumes, such as lupins, potato, sugar beet, pea, bean and soybean, and cruciferous plants (family Brassicaceae), such as cauliflower, rape seed, and the closely related model organism Arabidopsis thaliana. Thus, the transgenic plants and seeds of the invention include a broad range of plants, including, but not limited to, species from the genera Anacardium, Arachis, Asparagus, Atropa, Avena, Brassica, Citrus, Citrullus, Capsicum, Carthamus, Cocos, Coffea, Cucumis, Cucurbita, Daucus, Elaeis, Fragaria, Glycine, Gossypium, Helianthus, Heterocallis, Hordeum, Hyoscyamus, Lactuca, Linum, Lolium, Lupinus, Lycopersicon, Malus, Manihot, Majorana, Medicago, Nicotiana, Olea, Oryza, Panieum, Pannisetum, Persea, Phaseolus, Pistachia, Pisum, Pyrus, Prunus, Raphanus, Ricinus, Secale, Senecio, Sinapis, Solanum, Sorghum, Theobromus, Trigonella, Triticum, Vicia, Vitis, Vigna, and Zea.

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In alternative embodiments, the nucleic acids of the invention are expressed in plants which contain fiber cells, including, e.g., cotton, silk cotton tree (Kapok, Ceiba pentandra), desert willow, creosote bush, winterfat, balsa, ramie, kenaf, hemp, roselle, jute, sisal abaca and flax. In alternative embodiments, the transgenic plants of the invention can be members of the genus Gossypium, including members of any Gossypium species, such as G. arboreum; G. herbaceum, G. barbadense, and G. hirsutum.

The invention also provides for transgenic plants to be used for producing large amounts of the polypeptides (e.g., a xylanase or antibody) of the invention. For example, see Palmgren (1997) Trends Genet. 13:348; Chong (1997) Transgenic Res. 6:289-296 (producing human milk protein beta-casein in transgenic potato plants using an auxin-inducible, bidirectional mannopine synthase (mas1',2') promoter with Agrobacterium tumefaciens-mediated leaf disc transformation methods).

Using known procedures, one of skill can screen for plants of the invention by
detecting the increase or decrease of transgene mRNA or protein in transgenic plants. Means
for detecting and quantitation of mRNAs or proteins are well known in the art.

Polypeptides and peptides

In one aspect, the invention provides isolated or recombinant polypeptides having a sequence identity (e.g., at least about 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or complete (100%) sequence identity) to an exemplary sequence of the invention, e.g., proteins having a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20. 10 SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID 15 NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID 20 NO:128, SEQ ID NO:130, SEQ ID NO:132; SEQ ID NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; SEQ ID NO:142; SEQ ID NO:144; NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178. 25 SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, 30 SEQ ID NO:230, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, SEQ ID NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278,

SEQ ID NO:280, SEQ ID NO:282, SEQ ID NO:284, SEQ ID NO:286, SEO ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, 5 SEQ ID NO:320, SEQ ID NO:322, SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID NO:334, SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376, SEQ ID NO:378 or SEQ ID NO:380. In one aspect, the polypeptide has a xylanase activity, e.g., can hydrolyze a glycosidic bond in a polysaccharide, e.g., a xylan. In one aspect, the polypeptide has a xylanase activity comprising catalyzing hydrolysis of internal β-1,4-xylosidic linkages. In one aspect, the xylanase activity comprises an endo-1,4-beta-xylanase activity. In one aspect, the xylanase activity comprises hydrolyzing a xylan to produce a smaller molecular weight xylose and xylo-oligomer. In one aspect, the xylan comprises an arabinoxylan, such as a water soluble arabinoxylan.

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The polypeptides of the invention include xylanases in an active or inactive form. For example, the polypeptides of the invention include proproteins before "maturation" or processing of prepro sequences, e.g., by a proprotein-processing enzyme, such as a proprotein convertase to generate an "active" mature protein. The polypeptides of the invention include xylanases inactive for other reasons, e.g., before "activation" by a posttranslational processing event, e.g., an endo- or exo-peptidase or proteinase action, a phosphorylation event, an amidation, a glycosylation or a sulfation, a dimerization event, and the like. The polypeptides of the invention include all active forms, including active subsequences, e.g., catalytic domains or active sites, of the xylanase.

Methods for identifying "prepro" domain sequences and signal sequences are well known in the art, see, e.g., Van de Ven (1993) Crit. Rev. Oncog. 4(2):115-136. For example, to identify a prepro sequence, the protein is purified from the extracellular space and the N-terminal protein sequence is determined and compared to the unprocessed form.

The invention includes polypeptides with or without a signal sequence and/or a prepro sequence. The invention includes polypeptides with heterologous signal sequences and/or prepro sequences. The prepro sequence (including a sequence of the invention used as a heterologous prepro domain) can be located on the amino terminal or the carboxy terminal

end of the protein. The invention also includes isolated or recombinant signal sequences, prepro sequences and catalytic domains (e.g., "active sites") comprising sequences of the invention.

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The percent sequence identity can be over the full length of the polypeptide, or, the identity can be over a region of at least about 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700 or more residues. Polypeptides of the invention can also be shorter than the full length of exemplary polypeptides. In alternative aspects, the invention provides polypeptides (peptides, fragments) ranging in size between about 5 and the full length of a polypeptide, e.g., an enzyme, such as a xylanase; exemplary sizes being of about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, or more residues, e.g., contiguous residues of an exemplary xylanase of the invention.

Peptides of the invention (e.g., a subsequence of an exemplary polypeptide of the invention) can be useful as, e.g., labeling probes, antigens, toleragens, motifs, xylanase active sites (e.g., "catalytic domains"), signal sequences and/or prepro domains.

Polypeptides and peptides of the invention can be isolated from natural sources, be synthetic, or be recombinantly generated polypeptides. Peptides and proteins can be recombinantly expressed *in vitro* or *in vivo*. The peptides and polypeptides of the invention can be made and isolated using any method known in the art. Polypeptide and peptides of the invention can also be synthesized, whole or in part, using chemical methods well known in the art. See e.g., Caruthers (1980) Nucleic Acids Res. Symp. Ser. 215-223; Horn (1980) Nucleic Acids Res. Symp. Ser. 225-232; Banga, A.K., Therapeutic Peptides and Proteins, Formulation, Processing and Delivery Systems (1995) Technomic Publishing Co., Lancaster, PA. For example, peptide synthesis can be performed using various solid-phase techniques (see e.g., Roberge (1995) Science 269:202; Merrifield (1997) Methods Enzymol. 289:3-13) and automated synthesis may be achieved, e.g., using the ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

The peptides and polypeptides of the invention can also be glycosylated. The glycosylation can be added post-translationally either chemically or by cellular biosynthetic mechanisms, wherein the later incorporates the use of known glycosylation motifs, which can be native to the sequence or can be added as a peptide or added in the nucleic acid coding sequence. The glycosylation can be O-linked or N-linked.

The peptides and polypeptides of the invention, as defined above, include all "mimetic" and "peptidomimetic" forms. The terms "mimetic" and "peptidomimetic" refer to

a synthetic chemical compound which has substantially the same structural and/or functional characteristics of the polypeptides of the invention. The mimetic can be either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric molecule of partly natural peptide amino acids and partly non-natural analogs of amino acids. The mimetic can also incorporate any amount of natural amino acid conservative substitutions as long as such substitutions also do not substantially alter the mimetic's structure and/or activity. As with polypeptides of the invention which are conservative variants, routine experimentation will determine whether a mimetic is within the scope of the invention, i.e., that its structure and/or function is not substantially altered. Thus, in one aspect, a mimetic composition is within the scope of the invention if it has a xylanase activity.

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Polypeptide mimetic compositions of the invention can contain any combination of non-natural structural components. In alternative aspect, mimetic compositions of the invention include one or all of the following three structural groups: a) residue linkage groups other than the natural amide bond ("peptide bond") linkages; b) nonnatural residues in place of naturally occurring amino acid residues; or c) residues which induce secondary structural mimicry, i.e., to induce or stabilize a secondary structure, e.g., a beta turn, gamma turn, beta sheet, alpha helix conformation, and the like. For example, a polypeptide of the invention can be characterized as a mimetic when all or some of its residues are joined by chemical means other than natural peptide bonds. Individual peptidomimetic residues can be joined by peptide bonds, other chemical bonds or coupling means, such as, e.g., glutaraldehyde, N-hydroxysuccinimide esters, bifunctional maleimides, N,N'-dicyclohexylcarbodiimide (DCC) or N,N'-diisopropylcarbodiimide (DIC). Linking groups that can be an alternative to the traditional amide bond ("peptide bond") linkages include, e.g., ketomethylene (e.g., -C(=0)-CH₂- for -C(=0)-NH-), aminomethylene (CH₂-NH), ethylene, olefin (CH=CH), ether (CH₂-O), thioether (CH₂-S), tetrazole (CN₄-), thiazole, retroamide, thioamide, or ester (see, e.g., Spatola (1983) in Chemistry and Biochemistry of Amino Acids, Peptides and Proteins, Vol. 7, pp 267-357, "Peptide Backbone Modifications," Marcell Dekker, NY).

A polypeptide of the invention can also be characterized as a mimetic by containing all or some non-natural residues in place of naturally occurring amino acid residues. Non-natural residues are well described in the scientific and patent literature; a few exemplary non-natural compositions useful as mimetics of natural amino acid residues and guidelines are described below. Mimetics of aromatic amino acids can be generated by replacing by, e.g., D- or L- naphylalanine; D- or L- phenylglycine; D- or L-2 thieneylalanine;

D- or L-1, -2, 3-, or 4- pyreneylalanine; D- or L-3 thieneylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine; D-(trifluoromethyl)-phenylglycine; D-(trifluoromethyl)-phenylalanine; D-p-fluoro-phenylalanine; D- or L-p-biphenylphenylalanine; D- or L-p-methoxy-

biphenylphenylalanine; D- or L-2-indole(alkyl)alanines; and, D- or L-alkylainines, where alkyl can be substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, iso-popyl, iso-butyl, sec-isotyl, iso-pentyl, or a non-acidic amino acids. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings.

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Mimetics of acidic amino acids can be generated by substitution by, e.g., noncarboxylate amino acids while maintaining a negative charge; (phosphono)alanine; sulfated threonine. Carboxyl side groups (e.g., aspartyl or glutamyl) can also be selectively modified by reaction with carbodiimides (R'-N-C-N-R') such as, e.g., 1-cyclohexyl-3(2-morpholinyl-(4-ethyl) carbodiimide or 1-ethyl-3(4-azonia-4,4-dimetholpentyl) carbodiimide. Aspartyl or glutamyl can also be converted to asparaginyl and glutaminyl residues by reaction with ammonium ions. Mimetics of basic amino acids can be generated by substitution with, e.g., (in addition to lysine and arginine) the amino acids ornithine, citrulline, or (guanidino)-acetic acid, or (guanidino)alkyl-acetic acid, where alkyl is defined above. Nitrile derivative (e.g., containing the CN-moiety in place of COOH) can be substituted for asparagine or glutamine. Asparaginyl and glutaminyl residues can be deaminated to the corresponding aspartyl or glutamyl residues. Arginine residue mimetics can be generated by reacting arginyl with, e.g., one or more conventional reagents, including, e.g., phenylglyoxal, 2,3-butanedione, 1,2cyclo-hexanedione, or ninhydrin, preferably under alkaline conditions. Tyrosine residue mimetics can be generated by reacting tyrosyl with, e.g., aromatic diazonium compounds or tetranitromethane. N-acetylimidizol and tetranitromethane can be used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Cysteine residue mimetics can be generated by reacting cysteinyl residues with, e.g., alpha-haloacetates such as 2-chloroacetic acid or chloroacetamide and corresponding amines; to give carboxymethyl or carboxyamidomethyl derivatives. Cysteine residue mimetics can also be generated by reacting cysteinyl residues with, e.g., bromo-trifluoroacetone, alpha-bromo-beta-(5imidozoyl) propionic acid; chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide; methyl 2-pyridyl disulfide; p-chloromercuribenzoate; 2-chloromercuri-4 nitrophenol; or, chloro-7-nitrobenzo-oxa-1,3-diazole. Lysine mimetics can be generated (and amino terminal residues can be altered) by reacting lysinyl with, e.g., succinic or other

carboxylic acid anhydrides. Lysine and other alpha-amino-containing residue mimetics can also be generated by reaction with imidoesters, such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitro-benzenesulfonic acid, O-methylisourea, 2,4, pentanedione, and transamidase-catalyzed reactions with glyoxylate. Mimetics of methionine can be generated by reaction with, e.g., methionine sulfoxide. Mimetics of proline include, e.g., pipecolic acid, thiazolidine carboxylic acid, 3- or 4- hydroxy proline, dehydroproline, 3- or 4-methylproline, or 3,3,-dimethylproline. Histidine residue mimetics can be generated by reacting histidyl with, e.g., diethylprocarbonate or para-bromophenacyl bromide. Other mimetics include, e.g., those generated by hydroxylation of proline and lysine; phosphorylation of the hydroxyl groups of seryl or threonyl residues; methylation of the alpha-amino groups of lysine, arginine and histidine; acetylation of the N-terminal amine; methylation of main chain amide residues or substitution with N-methyl amino acids; or amidation of C-terminal carboxyl groups.

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A residue, e.g., an amino acid, of a polypeptide of the invention can also be replaced by an amino acid (or peptidomimetic residue) of the opposite chirality. Thus, any amino acid naturally occurring in the L-configuration (which can also be referred to as the R or S, depending upon the structure of the chemical entity) can be replaced with the amino acid of the same chemical structural type or a peptidomimetic, but of the opposite chirality, referred to as the D- amino acid, but also can be referred to as the R- or S- form.

The invention also provides methods for modifying the polypeptides of the invention by either natural processes, such as post-translational processing (e.g., phosphorylation, acylation, etc), or by chemical modification techniques, and the resulting modified polypeptides. Modifications can occur anywhere in the polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also a given polypeptide may have many types of modifications. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of a phosphatidylinositol, cross-linking cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristolyation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, and transfer-

RNA mediated addition of amino acids to protein such as arginylation. See, e.g., Creighton, T.E., Proteins – Structure and Molecular Properties 2nd Ed., W.H. Freeman and Company, New York (1993); Posttranslational Covalent Modification of Proteins, B.C. Johnson, Ed., Academic Press, New York, pp. 1-12 (1983).

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Solid-phase chemical peptide synthesis methods can also be used to synthesize the polypeptide or fragments of the invention. Such method have been known in the art since the early 1960's (Merrifield, R. B., J. Am. Chem. Soc., 85:2149-2154, 1963) (See also Stewart, J. M. and Young, J. D., Solid Phase Peptide Synthesis, 2nd Ed., Pierce Chemical Co., Rockford, Ill., pp. 11-12)) and have recently been employed in commercially available laboratory peptide design and synthesis kits (Cambridge Research Biochemicals). Such commercially available laboratory kits have generally utilized the teachings of H. M. Geysen et al, Proc. Natl. Acad. Sci., USA, 81:3998 (1984) and provide for synthesizing peptides upon the tips of a multitude of "rods" or "pins" all of which are connected to a single plate. When such a system is utilized, a plate of rods or pins is inverted and inserted into a second plate of corresponding wells or reservoirs, which contain solutions for attaching or anchoring an appropriate amino acid to the pin's or rod's tips. By repeating such a process step, i.e., inverting and inserting the rod's and pin's tips into appropriate solutions, amino acids are built into desired peptides. In addition, a number of available FMOC peptide synthesis systems are available. For example, assembly of a polypeptide or fragment can be carried out on a solid support using an Applied Biosystems, Inc. Model $431A^{\text{TM}}$ automated peptide synthesizer. Such equipment provides ready access to the peptides of the invention, either by direct synthesis or by synthesis of a series of fragments that can be coupled using other known techniques.

The invention includes xylanases of the invention with and without signal.

The polypeptide comprising a signal sequence of the invention can be a xylanase of the invention or another xylanase or another enzyme or other polypeptide.

The invention includes immobilized xylanases, anti-xylanase antibodies and fragments thereof. The invention provides methods for inhibiting xylanase activity, e.g., using dominant negative mutants or anti-xylanase antibodies of the invention. The invention includes heterocomplexes, e.g., fusion proteins, heterodimers, etc., comprising the xylanases of the invention.

Polypeptides of the invention can have a xylanase activity under various conditions, e.g., extremes in pH and/or temperature, oxidizing agents, and the like. The invention provides methods leading to alternative xylanase preparations with different

catalytic efficiencies and stabilities, e.g., towards temperature, oxidizing agents and changing wash conditions. In one aspect, xylanase variants can be produced using techniques of site-directed mutagenesis and/or random mutagenesis. In one aspect, directed evolution can be used to produce a great variety of xylanase variants with alternative specificities and stability.

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The proteins of the invention are also useful as research reagents to identify xylanase modulators, e.g., activators or inhibitors of xylanase activity. Briefly, test samples (compounds, broths, extracts, and the like) are added to xylanase assays to determine their ability to inhibit substrate cleavage. Inhibitors identified in this way can be used in industry and research to reduce or prevent undesired proteolysis. As with xylanases, inhibitors can be combined to increase the spectrum of activity.

The enzymes of the invention are also useful as research reagents to digest proteins or in protein sequencing. For example, the xylanases may be used to break polypeptides into smaller fragments for sequencing using, e.g. an automated sequencer.

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The invention also provides methods of discovering new xylanases using the nucleic acids, polypeptides and antibodies of the invention. In one aspect, phagemid libraries are screened for expression-based discovery of xylanases. In another aspect, lambda phage libraries are screened for expression-based discovery of xylanases. Screening of the phage or phagemid libraries can allow the detection of toxic clones; improved access to substrate; reduced need for engineering a host, by-passing the potential for any bias resulting from mass excision of the library; and, faster growth at low clone densities. Screening of phage or phagemid libraries can be in liquid phase or in solid phase. In one aspect, the invention provides screening in liquid phase. This gives a greater flexibility in assay conditions; additional substrate flexibility; higher sensitivity for weak clones; and ease of automation over solid phase screening.

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The invention provides screening methods using the proteins and nucleic acids of the invention and robotic automation to enable the execution of many thousands of biocatalytic reactions and screening assays in a short period of time, e.g., per day, as well as ensuring a high level of accuracy and reproducibility (see discussion of arrays, below). As a result, a library of derivative compounds can be produced in a matter of weeks. For further teachings on modification of molecules, including small molecules, see PCT/US94/09174.

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Another aspect of the invention is an isolated or purified polypeptide comprising the sequence of one of Group A nucleic acid sequences and sequences substantially identical thereto, or fragments comprising at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof. As discussed above, such

polypeptides may be obtained by inserting a nucleic acid encoding the polypeptide into a vector such that the coding sequence is operably linked to a sequence capable of driving the expression of the encoded polypeptide in a suitable host cell. For example, the expression vector may comprise a promoter, a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

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Another aspect of the invention is polypeptides or fragments thereof which have at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more than about 95% homology to one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or a fragment comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof. Homology may be determined using any of the programs described above which aligns the polypeptides or fragments being compared and determines the extent of amino acid identity or similarity between them. It will be appreciated that amino acid "homology" includes conservative amino acid substitutions such as those described above.

The polypeptides or fragments having homology to one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or a fragment comprising at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof may be obtained by isolating the nucleic acids encoding them using the techniques described above.

Alternatively, the homologous polypeptides or fragments may be obtained through biochemical enrichment or purification procedures. The sequence of potentially homologous polypeptides or fragments may be determined by xylan hydrolase digestion, gel electrophoresis and/or microsequencing. The sequence of the prospective homologous polypeptide or fragment can be compared to one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or a fragment comprising at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof using any of the programs described above.

Another aspect of the invention is an assay for identifying fragments or variants of Group B amino acid sequences and sequences substantially identical thereto, which retain the enzymatic function of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto. For example the fragments or variants of said polypeptides, may be used to catalyze biochemical reactions, which indicate that the fragment

or variant retains the enzymatic activity of the polypeptides in the Group B amino acid sequences.

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The assay for determining if fragments of variants retain the enzymatic activity of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto includes the steps of: contacting the polypeptide fragment or variant with a substrate molecule under conditions which allow the polypeptide fragment or variant to function and detecting either a decrease in the level of substrate or an increase in the level of the specific reaction product of the reaction between the polypeptide and substrate.

The polypeptides of Group B amino acid sequences and sequences substantially identical thereto or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof may be used in a variety of applications. For example, the polypeptides or fragments thereof may be used to catalyze biochemical reactions. In accordance with one aspect of the invention, there is provided a process for utilizing the polypeptides of Group B amino acid sequences and sequences substantially identical thereto or polynucleotides encoding such polypeptides for hydrolyzing glycosidic linkages. In such procedures, a substance containing a glycosidic linkage (e.g., a starch) is contacted with one of the polypeptides of Group B amino acid sequences, or sequences substantially identical thereto under conditions which facilitate the hydrolysis of the glycosidic linkage.

The present invention exploits the unique catalytic properties of enzymes. Whereas the use of biocatalysts (i.e., purified or crude enzymes, non-living or living cells) in chemical transformations normally requires the identification of a particular biocatalyst that reacts with a specific starting compound, the present invention uses selected biocatalysts and reaction conditions that are specific for functional groups that are present in many starting compounds, such as small molecules. Each biocatalyst is specific for one functional group, or several related functional groups and can react with many starting compounds containing this functional group.

The biocatalytic reactions produce a population of derivatives from a single starting compound. These derivatives can be subjected to another round of biocatalytic reactions to produce a second population of derivative compounds. Thousands of variations of the original small molecule or compound can be produced with each iteration of biocatalytic derivatization.

Enzymes react at specific sites of a starting compound without affecting the rest of the molecule, a process which is very difficult to achieve using traditional chemical

methods. This high degree of biocatalytic specificity provides the means to identify a single active compound within the library. The library is characterized by the series of biocatalytic reactions used to produce it, a so called "biosynthetic history". Screening the library for biological activities and tracing the biosynthetic history identifies the specific reaction sequence producing the active compound. The reaction sequence is repeated and the structure of the synthesized compound determined. This mode of identification, unlike other synthesis and screening approaches, does not require immobilization technologies and compounds can be synthesized and tested free in solution using virtually any type of screening assay. It is important to note, that the high degree of specificity of enzyme reactions on functional groups allows for the "tracking" of specific enzymatic reactions that make up the biocatalytically produced library.

Many of the procedural steps are performed using robotic automation enabling the execution of many thousands of biocatalytic reactions and screening assays per day as well as ensuring a high level of accuracy and reproducibility. As a result, a library of derivative compounds can be produced in a matter of weeks which would take years to produce using current chemical methods.

In a particular aspect, the invention provides a method for modifying small molecules, comprising contacting a polypeptide encoded by a polynucleotide described herein or enzymatically active fragments thereof with a small molecule to produce a modified small molecule. A library of modified small molecules is tested to determine if a modified small molecule is present within the library which exhibits a desired activity. A specific biocatalytic reaction which produces the modified small molecule of desired activity is identified by systematically eliminating each of the biocatalytic reactions used to produce a portion of the library and then testing the small molecules produced in the portion of the library for the presence or absence of the modified small molecule with the desired activity. The specific biocatalytic reactions which produce the modified small molecule of desired activity is optionally repeated. The biocatalytic reactions are conducted with a group of biocatalysts that react with distinct structural moieties found within the structure of a small molecule, each biocatalyst is specific for one structural moiety or a group of related structural moieties; and each biocatalyst reacts with many different small molecules which contain the distinct structural moiety.

Xylanase signal sequences, prepro and catalytic domains

The invention provides xylanase signal sequences (e.g., signal peptides (SPs)), prepro domains and catalytic domains (CDs). The SPs, prepro domains and/or CDs of the invention can be isolated or recombinant peptides or can be part of a fusion protein, e.g., as a heterologous domain in a chimeric protein. The invention provides nucleic acids encoding these catalytic domains (CDs), prepro domains and signal sequences (SPs, e.g., a peptide having a sequence comprising/ consisting of amino terminal residues of a polypeptide of the invention). In one aspect, the invention provides a signal sequence comprising a peptide comprising/ consisting of a sequence as set forth in residues 1 to 15, 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30, 1 to 31, 1 to 32, 1 to 33, 1 to 34, 1 to 35, 1 to 36, 1 to 37, 1 to 38, 1 to 39, 1 to 40, 1 to 41, 1 to 42, 1 to 43, 1 to 44 of a polypeptide of the invention.

In one aspect, the invention provides a signal sequence comprising a peptide comprising/ consisting of a sequence as set forth in Table 4 below. For example, in reading Table 4, the invention provides a signal sequence comprising/ consisting of residues 1 to 23 of SEQ ID NO:102 (encoded by SEQ ID NO:101), a signal sequence comprising/ consisting of residues 1 to 41 of SEQ ID NO:104 (encoded by SEQ ID NO:103), etc.

Table 4: exemplary signal sequences of the invention

Signal sequence (amino acid SEQ ID NO: positions) 101, 102 1-23 103, 104 1-41 105, 106 1-22 109, 110 1-26 11, 12 1-28 113, 114 1-28 119, 120 1-33 121, 122 1-20 123, 124 1-20 131, 132 1-26 135, 136 1-25 139, 140 1-24 141, 142 1-25 143, 144 1-32 147, 148 1-28 149, 150 1-18 15, 16 1-20 151, 152 1-21 153, 154 1-16

155, 156 1-21

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157, 158 1-2	29
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^{159, 160 1-23}

- 167, 168 1-36
- 169, 170 1-24
- 17, 18 1-31
- 171, 172 1-29
- 173, 174 1-22
- 175, 176 1-27
- 177, 178 1-26
- 179, 180 1-19
- 181, 182 1-25
- 183, 184 1-32
- 185, 186 1-27
- 187, 188 1-28
- 19, 20 1-29
- 191, 192 1-27
- 193, 194 1-21
- 195, 196 1-23
- 197, 198 1-28
- 199, 200 1-30
- 203, 204 1-30
- 205, 206 1-29
- 207, 208 1-27
- 209, 210 1-25 21, 22 1-28
- 211, 212 1-29
- 215, 216 1-31
- 217, 218 1-29
- 219, 220 1-23
- 221, 222 1-24
- 223, 224 1-28
- 225, 226 1-25
- 227, 228 1-39
- 229, 230 1-28
- 23, 24 1-29
- 231, 232 1-41
- 233, 234 1-26
- 235, 236 1-28
- 237, 238 1-32
- 239, 240 1-30
- 241, 242 1-28
- 243, 244 1-33
- 245, 246 1-32
- 249, 250 1-33
- 253, 254 1-24
- 255, 256 1-51
- 259, 260 1-24 261, 262 1-26
- 263, 264 1-29

^{161, 162 1-32}

267 260	1-30
267, 268	
27, 28	1-27
271, 272	1-22
273, 274	1-74
277, 278	1-19
279, 280	1-22
283, 284	1-28
	1-23
287, 288	
289, 290	1-22
295, 296	1-26
299, 300	1-24
301, 302	1-28
303, 304	1-74
305, 306	1-32
309, 310	1-20
311, 312	1-33
313, 314	1-22
315, 316	1-28
319, 320	1-27
325, 326	1-27
-	
327, 328	1-29
329, 330	1-35
33, 34	1-23
331, 332	1-28
333, 334	1-30
335, 336	1-50
339, 340	1-23
341, 342	1-45
347, 348	1-20
349, 350	1-20
351, 352	1-73
353, 354	1-18
	1-21
355, 356	
357, 358	1-25
359, 360	1-31
361, 362	1-26
365, 366	1-65
367, 368	1-23
369, 370	1-27
39, 40	1-24
41, 42	1-37
45, 46	1-25
47, 48	1-26
5, 6	1-47
51, 52	1-30
53, 54	1-37
55, 56	1-24
57, 58	1-22
59, 60	1-21
63, 64	1-20
65, 66	1-22
67, 68	1-28

69, 70 1-25 7, 8 1-57 73, 74 1-21 75, 76 1-22 77, 78 1-27 79, 80 1-36 83, 84 1-30 87, 88 1-29 89, 90 1-40 9, 10 1-36 95, 96 1-24 99, 100 1-33

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The xylanase signal sequences (SPs) and/or prepro sequences of the invention can be isolated peptides, or, sequences joined to another xylanase or a non-xylanase polypeptide, e.g., as a fusion (chimeric) protein. In one aspect, the invention provides polypeptides comprising xylanase signal sequences of the invention. In one aspect, polypeptides comprising xylanase signal sequences SPs and/or prepro of the invention comprise sequences heterologous to a xylanase of the invention (e.g., a fusion protein comprising an SP and/or prepro of the invention and sequences from another xylanase or a non-xylanase protein). In one aspect, the invention provides xylanases of the invention with heterologous SPs and/or prepro sequences, e.g., sequences with a yeast signal sequence. A xylanase of the invention can comprise a heterologous SP and/or prepro in a vector, e.g., a pPIC series vector (Invitrogen, Carlsbad, CA).

In one aspect, SPs and/or prepro sequences of the invention are identified following identification of novel xylanase polypeptides. The pathways by which proteins are sorted and transported to their proper cellular location are often referred to as protein targeting pathways. One of the most important elements in all of these targeting systems is a short amino acid sequence at the amino terminus of a newly synthesized polypeptide called the signal sequence. This signal sequence directs a protein to its appropriate location in the cell and is removed during transport or when the protein reaches its final destination. Most lysosomal, membrane, or secreted proteins have an amino-terminal signal sequence that marks them for translocation into the lumen of the endoplasmic reticulum. More than 100 signal sequences for proteins in this group have been determined. The signal sequences can vary in length from 13 to 36 amino acid residues. Various methods of recognition of signal sequences are known to those of skill in the art. For example, in one aspect, novel xylanase signal peptides are identified by a method referred to as SignalP. SignalP uses a combined neural network which recognizes both signal peptides and their cleavage sites. (Nielsen, et

al., "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites." Protein Engineering, vol. 10, no. 1, p. 1-6 (1997).

It should be understood that in some aspects xylanases of the invention may not have SPs and/or prepro sequences, or "domains." In one aspect, the invention provides the xylanases of the invention lacking all or part of an SP and/or a prepro domain. In one aspect, the invention provides a nucleic acid sequence encoding a signal sequence (SP) and/or prepro from one xylanase operably linked to a nucleic acid sequence of a different xylanase or, optionally, a signal sequence (SPs) and/or prepro domain from a non-xylanase protein may be desired.

The invention also provides isolated or recombinant polypeptides comprising signal sequences (SPs), prepro domain and/or catalytic domains (CDs) of the invention and heterologous sequences. The heterologous sequences are sequences not naturally associated (e.g., to a xylanase) with an SP, prepro domain and/or CD. The sequence to which the SP, prepro domain and/or CD are not naturally associated can be on the SP's, prepro domain and/or CD's amino terminal end, carboxy terminal end, and/or on both ends of the SP and/or CD. In one aspect, the invention provides an isolated or recombinant polypeptide comprising (or consisting of) a polypeptide comprising a signal sequence (SP), prepro domain and/or catalytic domain (CD) of the invention with the proviso that it is not associated with any sequence to which it is naturally associated (e.g., a xylanase sequence). Similarly in one aspect, the invention provides isolated or recombinant nucleic acids encoding these polypeptides. Thus, in one aspect, the isolated or recombinant nucleic acid of the invention comprises coding sequence for a signal sequence (SP), prepro domain and/or catalytic domain (CD) of the invention and a heterologous sequence (i.e., a sequence not naturally associated with the a signal sequence (SP), prepro domain and/or catalytic domain (CD) of the invention). The heterologous sequence can be on the 3' terminal end, 5' terminal end, and/or on both ends of the SP, prepro domain and/or CD coding sequence.

Hybrid (chimeric) xylanases and peptide libraries

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In one aspect, the invention provides hybrid xylanases and fusion proteins, including peptide libraries, comprising sequences of the invention. The peptide libraries of the invention can be used to isolate peptide modulators (e.g., activators or inhibitors) of targets, such as xylanase substrates, receptors, enzymes. The peptide libraries of the invention can be used to identify formal binding partners of targets, such as ligands, e.g., cytokines, hormones and the like. In one aspect, the invention provides chimeric proteins

comprising a signal sequence (SP), prepro domain and/or catalytic domain (CD) of the invention or a combination thereof and a heterologous sequence (see above).

In one aspect, the fusion proteins of the invention (e.g., the peptide moiety) are conformationally stabilized (relative to linear peptides) to allow a higher binding affinity for targets. The invention provides fusions of xylanases of the invention and other peptides, including known and random peptides. They can be fused in such a manner that the structure of the xylanases is not significantly perturbed and the peptide is metabolically or structurally conformationally stabilized. This allows the creation of a peptide library that is easily monitored both for its presence within cells and its quantity.

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Amino acid sequence variants of the invention can be characterized by a predetermined nature of the variation, a feature that sets them apart from a naturally occurring form, e.g., an allelic or interspecies variation of a xylanase sequence. In one aspect, the variants of the invention exhibit the same qualitative biological activity as the naturally occurring analogue. Alternatively, the variants can be selected for having modified characteristics. In one aspect, while the site or region for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, in order to optimize the performance of a mutation at a given site, random mutagenesis may be conducted at the target codon or region and the expressed xylanase variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, as discussed herein for example, M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants can be done using, e.g., assays of xylan hydrolysis. In alternative aspects, amino acid substitutions can be single residues; insertions can be on the order of from about 1 to 20 amino acids, although considerably larger insertions can be done. Deletions can range from about 1 to about 20, 30, 40, 50, 60, 70 residues or more. To obtain a final derivative with the optimal properties, substitutions, deletions, insertions or any combination thereof may be used. Generally, these changes are done on a few amino acids to minimize the alteration of the molecule. However, larger changes may be tolerated in certain circumstances.

The invention provides xylanases where the structure of the polypeptide backbone, the secondary or the tertiary structure, e.g., an alpha-helical or beta-sheet structure, has been modified. In one aspect, the charge or hydrophobicity has been modified. In one aspect, the bulk of a side chain has been modified. Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative. For example, substitutions can be made which more significantly affect: the structure of the

polypeptide backbone in the area of the alteration, for example a alpha-helical or a beta-sheet structure; a charge or a hydrophobic site of the molecule, which can be at an active site; or a side chain. The invention provides substitutions in polypeptide of the invention where (a) a hydrophilic residues, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g. lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g. glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g. phenylalanine, is substituted for (or by) one not having a side chain, e.g. glycine. The variants can exhibit the same qualitative biological activity (i.e. xylanase activity) although variants can be selected to modify the characteristics of the xylanases as needed.

In one aspect, xylanases of the invention comprise epitopes or purification tags, signal sequences or other fusion sequences, etc. In one aspect, the xylanases of the invention can be fused to a random peptide to form a fusion polypeptide. By "fused" or "operably linked" herein is meant that the random peptide and the xylanase are linked together, in such a manner as to minimize the disruption to the stability of the xylanase structure, e.g., it retains xylanase activity. The fusion polypeptide (or fusion polynucleotide encoding the fusion polypeptide) can comprise further components as well, including multiple peptides at multiple loops.

In one aspect, the peptides and nucleic acids encoding them are randomized, either fully randomized or they are biased in their randomization, e.g. in nucleotide/residue frequency generally or per position. "Randomized" means that each nucleic acid and peptide consists of essentially random nucleotides and amino acids, respectively. In one aspect, the nucleic acids which give rise to the peptides can be chemically synthesized, and thus may incorporate any nucleotide at any position. Thus, when the nucleic acids are expressed to form peptides, any amino acid residue may be incorporated at any position. The synthetic process can be designed to generate randomized nucleic acids, to allow the formation of all or most of the possible combinations over the length of the nucleic acid, thus forming a library of randomized nucleic acids. The library can provide a sufficiently structurally diverse population of randomized expression products to affect a probabilistically sufficient range of cellular responses to provide one or more cells exhibiting a desired response. Thus, the invention provides an interaction library large enough so that at least one of its members will have a structure that gives it affinity for some molecule, protein, or other factor.

Xylanases are multidomain enzymes that consist optionally of a signal peptide, a carbohydrate binding module, a xylanase catalytic domain, a linker and/or another catalytic domain.

The invention provides a means for generating chimeric polypeptides which may encode biologically active hybrid polypeptides (e.g., hybrid xylanases). In one aspect, the original polynucleotides encode biologically active polypeptides. The method of the invention produces new hybrid polypeptides by utilizing cellular processes which integrate the sequence of the original polynucleotides such that the resulting hybrid polynucleotide encodes a polypeptide demonstrating activities derived from the original biologically active polypeptides. For example, the original polynucleotides may encode a particular enzyme from different microorganisms. An enzyme encoded by a first polynucleotide from one organism or variant may, for example, function effectively under a particular environmental condition, e.g. high salinity. An enzyme encoded by a second polynucleotide from a different organism or variant may function effectively under a different environmental condition, such as extremely high temperatures. A hybrid polynucleotide containing sequences from the first and second original polynucleotides may encode an enzyme which exhibits characteristics of both enzymes encoded by the original polynucleotides. Thus, the enzyme encoded by the hybrid polynucleotide may function effectively under environmental conditions shared by each of the enzymes encoded by the first and second polynucleotides, e.g., high salinity and extreme temperatures.

Enzymes encoded by the polynucleotides of the invention include, but are not limited to, hydrolases, such as xylanases. Glycosidase hydrolases were first classified into families in 1991, see, e.g., Henrissat (1991) Biochem. J. 280:309-316. Since then, the classifications have been continually updated, see, e.g., Henrissat (1993) Biochem. J. 293:781-788; Henrissat (1996) Biochem. J. 316:695-696; Henrissat (2000) Plant Physiology 124:1515-1519. There are 87 identified families of glycosidase hydrolases. In one aspect, the xylanases of the invention may be categorized in families 8, 10, 11, 26 and 30. In one aspect, the invention also provides xylanase-encoding nucleic acids with a common novelty in that they are derived from a common family, e.g., family 5, 6, 8, 10, 11, 26 or 30, as set forth in Table 5, below.

<u>Table 5</u>
SEQ ID FAMILY
9, 10 8
1, 2 8

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5, 6	8
7, 8	8
99, 100	10
11, 12	10
127, 128	10
27, 28	10
97, 98	10
45, 46	10
141, 142	10
107, 108	10
129, 130	10
93, 94	10
63, 64	10
25, 26	10
49, 50	10
67, 68	10
85, 86	10
29, 30	10
51, 52	10
35, 36	10
147, 148	10
119, 120	10
123, 124	10
249, 250	10
149, 150	10
83, 84	10
43, 44	10
133, 134	10
113, 114	10
105, 106	10
75, 76	10
111, 112	10
117, 118	10
115, 116	10
125, 126	10
137, 138	10
135, 136	10
69, 70	10
89, 90	10
31, 32	10
13, 14	10
65, 66	10
57, 58	10
77, 78	10
73, 74	10
109, 110	10
59, 60	10
71, 72	10
139, 140	10
55, 56	10
15, 16	10

131, 132	10
95, 96	10
101, 102	10
39, 40	10
143, 144	10
103, 104	10
17, 18	10
53, 54	10
21, 22	10
151, 152	10
23, 24	10
121, 122	10
41, 42	
	10
47, 48	10
247, 248	10
33, 34	10
19, 20	10
87, 88	10
81, 82	
	10
91, 92	10
61, 62	10
37, 38	10
79, 80	10
231, 232	11
157, 158	11
189, 190	11
167, 168	11
207, 208	11
251, 252	11
213, 214	11
177, 178	11
187, 188	11
205, 206	11
211, 212	11
197, 198	11
209, 210	11
185, 186	11
229, 230	11
223, 224	11
179, 180	11
193, 194	11
173, 174	11
217, 218	11
*	
153, 154	11
219, 220	11
183, 184	11
253, 254	11
199, 200	11
255, 256	11
155, 156	11
169, 170	
103, 170	11

195, 196	11
215, 216	
	11
191, 192	11
175, 176	11
161, 162	11
221, 222	11
225, 226	11
163, 164	11
159, 160	11
233, 234	11
171, 172	11
203, 204	11
181, 182	11
227, 228	11
165, 166	11
257, 258	
257, 256	26
237, 238	30
241, 242	30
239, 240	30
245, 246	30
235, 236	30
313, 314	30
345, 346	10
321, 322	10
323, 324	10
315, 316	10
201, 202	10
265, 266	10
145, 146	10
287, 288	10
293, 294	10
351, 352	10
311, 312	10
279, 280	10
289, 290	10
283, 284	10
373, 374	10
337, 338	10
371, 372	10
291, 292	10
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A hybrid polypeptide resulting from the method of the invention may exhibit specialized enzyme activity not displayed in the original enzymes. For example, following recombination and/or reductive reassortment of polynucleotides encoding hydrolase activities, the resulting hybrid polypeptide encoded by a hybrid polynucleotide can be screened for specialized hydrolase activities obtained from each of the original enzymes, i.e. the type of bond on which the hydrolase acts and the temperature at which the hydrolase functions. Thus, for example, the hydrolase may be screened to ascertain those chemical functionalities which distinguish the hybrid hydrolase from the original hydrolases, such as:

(a) amide (peptide bonds), i.e., xylanases; (b) ester bonds, i.e., esterases and lipases; (c) acetals, i.e., glycosidases and, for example, the temperature, pH or salt concentration at which the hybrid polypeptide functions.

Sources of the original polynucleotides may be isolated from individual organisms ("isolates"), collections of organisms that have been grown in defined media ("enrichment cultures"), or, uncultivated organisms ("environmental samples"). The use of a culture-independent approach to derive polynucleotides encoding novel bioactivities from environmental samples is most preferable since it allows one to access untapped resources of biodiversity.

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"Environmental libraries" are generated from environmental samples and represent the collective genomes of naturally occurring organisms archived in cloning vectors that can be propagated in suitable prokaryotic hosts. Because the cloned DNA is initially extracted directly from environmental samples, the libraries are not limited to the small fraction of prokaryotes that can be grown in pure culture. Additionally, a normalization of the environmental DNA present in these samples could allow more equal representation of the DNA from all of the species present in the original sample. This can dramatically increase the efficiency of finding interesting genes from minor constituents of the sample which may be under-represented by several orders of magnitude compared to the dominant species.

For example, gene libraries generated from one or more uncultivated microorganisms are screened for an activity of interest. Potential pathways encoding bioactive molecules of interest are first captured in prokaryotic cells in the form of gene expression libraries. Polynucleotides encoding activities of interest are isolated from such libraries and introduced into a host cell. The host cell is grown under conditions which promote recombination and/or reductive reassortment creating potentially active biomolecules with novel or enhanced activities.

Additionally, subcloning may be performed to further isolate sequences of interest. In subcloning, a portion of DNA is amplified, digested, generally by restriction enzymes, to cut out the desired sequence, the desired sequence is ligated into a recipient vector and is amplified. At each step in subcloning, the portion is examined for the activity of interest, in order to ensure that DNA that encodes the structural protein has not been excluded. The insert may be purified at any step of the subcloning, for example, by gel electrophoresis prior to ligation into a vector or where cells containing the recipient vector and cells not containing the recipient vector are placed on selective media containing, for example, an antibiotic, which will kill the cells not containing the recipient vector. Specific methods of subcloning cDNA inserts into vectors are well-known in the art (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press

(1989)). In another aspect, the enzymes of the invention are subclones. Such subclones may differ from the parent clone by, for example, length, a mutation, a tag or a label.

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In one aspect, the signal sequences of the invention are identified following identification of novel xylanase polypeptides. The pathways by which proteins are sorted and transported to their proper cellular location are often referred to as protein targeting pathways. One of the most important elements in all of these targeting systems is a short amino acid sequence at the amino terminus of a newly synthesized polypeptide called the signal sequence. This signal sequence directs a protein to its appropriate location in the cell and is removed during transport or when the protein reaches its final destination. Most lysosomal, membrane, or secreted proteins have an amino-terminal signal sequence that marks them for translocation into the lumen of the endoplasmic reticulum. More than 100 signal sequences for proteins in this group have been determined. The sequences vary in length from 13 to 36 amino acid residues. Various methods of recognition of signal sequences are known to those of skill in the art. In one aspect, the peptides are identified by a method referred to as SignalP. SignalP uses a combined neural network which recognizes both signal peptides and their cleavage sites. See, e.g., Nielsen (1997) "Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites." Protein Engineering, vol. 10, no. 1, p. 1-6. It should be understood that some of the xylanases of the invention may or may not contain signal sequences. It may be desirable to include a nucleic acid sequence encoding a signal sequence from one xylanase operably linked to a nucleic acid sequence of a different xylanase or, optionally, a signal sequence from a non-xylanase protein may be desired.

The microorganisms from which the polynucleotide may be prepared include prokaryotic microorganisms, such as Eubacteria and Archaebacteria and lower eukaryotic microorganisms such as fungi, some algae and protozoa. Polynucleotides may be isolated from environmental samples in which case the nucleic acid may be recovered without culturing of an organism or recovered from one or more cultured organisms. In one aspect, such microorganisms may be extremophiles, such as hyperthermophiles, psychrophiles, psychrophiles, psychrotrophs, halophiles, barophiles and acidophiles. Polynucleotides encoding enzymes isolated from extremophilic microorganisms can be used. Such enzymes may function at temperatures above 100°C in terrestrial hot springs and deep sea thermal vents, at temperatures below 0°C in arctic waters, in the saturated salt environment of the Dead Sea, at pH values around 0 in coal deposits and geothermal sulfur-rich springs, or at pH values greater than 11 in sewage sludge. For example, several esterases and lipases cloned and

expressed from extremophilic organisms show high activity throughout a wide range of temperatures and pHs.

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Polynucleotides selected and isolated as hereinabove described are introduced into a suitable host cell. A suitable host cell is any cell which is capable of promoting recombination and/or reductive reassortment. The selected polynucleotides are preferably already in a vector which includes appropriate control sequences. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or preferably, the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis *et al.*, 1986).

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as *E. coli*, *Streptomyces*, *Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila S2* and *Spodoptera Sf9*; animal cells such as CHO, COS or Bowes melanoma; adenoviruses; and plant cells. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

With particular references to various mammalian cell culture systems that can be employed to express recombinant protein, examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described in "SV40-transformed simian cells support the replication of early SV40 mutants" (Gluzman, 1981) and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

In another aspect, it is envisioned the method of the present invention can be used to generate novel polynucleotides encoding biochemical pathways from one or more operons or gene clusters or portions thereof. For example, bacteria and many eukaryotes have a coordinated mechanism for regulating genes whose products are involved in related processes. The genes are clustered, in structures referred to as "gene clusters," on a single chromosome and are transcribed together under the control of a single regulatory sequence, including a single promoter which initiates transcription of the entire cluster. Thus, a gene cluster is a group of adjacent genes that are either identical or related, usually as to their function. An example of a biochemical pathway encoded by gene clusters are polyketides.

Gene cluster DNA can be isolated from different organisms and ligated into vectors, particularly vectors containing expression regulatory sequences which can control and regulate the production of a detectable protein or protein-related array activity from the ligated gene clusters. Use of vectors which have an exceptionally large capacity for exogenous DNA introduction are particularly appropriate for use with such gene clusters and are described by way of example herein to include the f-factor (or fertility factor) of E. coli. This f-factor of E. coli is a plasmid which affects high-frequency transfer of itself during conjugation and is ideal to achieve and stably propagate large DNA fragments, such as gene clusters from mixed microbial samples. One aspect of the invention is to use cloning vectors, referred to as "fosmids" or bacterial artificial chromosome (BAC) vectors. These are derived from E. coli f-factor which is able to stably integrate large segments of genomic DNA. When integrated with DNA from a mixed uncultured environmental sample, this makes it possible to achieve large genomic fragments in the form of a stable "environmental DNA library." Another type of vector for use in the present invention is a cosmid vector. Cosmid vectors were originally designed to clone and propagate large segments of genomic DNA. Cloning into cosmid vectors is described in detail in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press (1989). Once ligated into an appropriate vector, two or more vectors containing different polyketide synthase gene clusters can be introduced into a suitable host cell. Regions of partial sequence homology shared by the gene clusters will promote processes which result in sequence reorganization resulting in a hybrid gene cluster. The novel hybrid gene cluster can then be screened for enhanced activities not found in the original gene clusters.

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Therefore, in a one aspect, the invention relates to a method for producing a biologically active hybrid polypeptide and screening such a polypeptide for enhanced activity by:

- introducing at least a first polynucleotide in operable linkage and a second
 polynucleotide in operable linkage, the at least first polynucleotide and second
 polynucleotide sharing at least one region of partial sequence homology, into a
 suitable host cell;
- 2) growing the host cell under conditions which promote sequence reorganization resulting in a hybrid polynucleotide in operable linkage;
- 3) expressing a hybrid polypeptide encoded by the hybrid polynucleotide;
- 4) screening the hybrid polypeptide under conditions which promote identification of enhanced biological activity; and

5) isolating the a polynucleotide encoding the hybrid polypeptide.

Methods for screening for various enzyme activities are known to those of skill in the art and are discussed throughout the present specification. Such methods may be employed when isolating the polypeptides and polynucleotides of the invention.

5 Screening Methodologies and "On-line" Monitoring Devices

In practicing the methods of the invention, a variety of apparatus and methodologies can be used to in conjunction with the polypeptides and nucleic acids of the invention, e.g., to screen polypeptides for xylanase activity (e.g., assays such as hydrolysis of casein in zymograms, the release of fluorescence from gelatin, or the release of p-nitroanalide from various small peptide substrates), to screen compounds as potential modulators, e.g., activators or inhibitors, of a xylanase activity, for antibodies that bind to a polypeptide of the invention, for nucleic acids that hybridize to a nucleic acid of the invention, to screen for cells expressing a polypeptide of the invention and the like. In addition to the array formats described in detail below for screening samples, alternative formats can also be used to practice the methods of the invention. Such formats include, for example, mass spectrometers, chromatographs, e.g., high-throughput HPLC and other forms of liquid chromatography, and smaller formats, such as 1536-well plates, 384-well plates and so on. High throughput screening apparatus can be adapted and used to practice the methods of the invention, see, e.g., U.S. Patent Application No. 20020001809.

Capillary Arrays

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Nucleic acids or polypeptides of the invention can be immobilized to or applied to an array. Arrays can be used to screen for or monitor libraries of compositions (e.g., small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide of the invention. Capillary arrays, such as the GIGAMATRIXTM, Diversa Corporation, San Diego, CA; and arrays described in, e.g., U.S. Patent Application No. 20020080350 A1; WO 0231203 A; WO 0244336 A, provide an alternative apparatus for holding and screening samples. In one aspect, the capillary array includes a plurality of capillaries formed into an array of adjacent capillaries, wherein each capillary comprises at least one wall defining a lumen for retaining a sample. The lumen may be cylindrical, square, hexagonal or any other geometric shape so long as the walls form a lumen for retention of a liquid or sample. The capillaries of the capillary array can be held together in close proximity to form a planar structure. The capillaries can be bound together, by being fused (e.g., where the capillaries are made of glass), glued, bonded, or clamped side-

by-side. Additionally, the capillary array can include interstitial material disposed between adjacent capillaries in the array, thereby forming a solid planar device containing a plurality of through-holes.

A capillary array can be formed of any number of individual capillaries, for example, a range from 100 to 4,000,000 capillaries. Further, a capillary array having about 100,000 or more individual capillaries can be formed into the standard size and shape of a Microtiter® plate for fitment into standard laboratory equipment. The lumens are filled manually or automatically using either capillary action or microinjection using a thin needle. Samples of interest may subsequently be removed from individual capillaries for further analysis or characterization. For example, a thin, needle-like probe is positioned in fluid communication with a selected capillary to either add or withdraw material from the lumen.

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In a single-pot screening assay, the assay components are mixed yielding a solution of interest, prior to insertion into the capillary array. The lumen is filled by capillary action when at least a portion of the array is immersed into a solution of interest. Chemical or biological reactions and/or activity in each capillary are monitored for detectable events. A detectable event is often referred to as a "hit", which can usually be distinguished from "non-hit" producing capillaries by optical detection. Thus, capillary arrays allow for massively parallel detection of "hits".

In a multi-pot screening assay, a polypeptide or nucleic acid, e.g., a ligand, can be introduced into a first component, which is introduced into at least a portion of a capillary of a capillary array. An air bubble can then be introduced into the capillary behind the first component. A second component can then be introduced into the capillary, wherein the second component is separated from the first component by the air bubble. The first and second components can then be mixed by applying hydrostatic pressure to both sides of the capillary array to collapse the bubble. The capillary array is then monitored for a detectable event resulting from reaction or non-reaction of the two components.

In a binding screening assay, a sample of interest can be introduced as a first liquid labeled with a detectable particle into a capillary of a capillary array, wherein the lumen of the capillary is coated with a binding material for binding the detectable particle to the lumen. The first liquid may then be removed from the capillary tube, wherein the bound detectable particle is maintained within the capillary, and a second liquid may be introduced into the capillary tube. The capillary is then monitored for a detectable event resulting from reaction or non-reaction of the particle with the second liquid.

Arrays, or "Biochips"

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Nucleic acids or polypeptides of the invention can be immobilized to or applied to an array. Arrays can be used to screen for or monitor libraries of compositions (e.g., small molecules, antibodies, nucleic acids, etc.) for their ability to bind to or modulate the activity of a nucleic acid or a polypeptide of the invention. For example, in one aspect of the invention, a monitored parameter is transcript expression of a xylanase gene. One or more, or, all the transcripts of a cell can be measured by hybridization of a sample comprising transcripts of the cell, or, nucleic acids representative of or complementary to transcripts of a cell, by hybridization to immobilized nucleic acids on an array, or "biochip." By using an "array" of nucleic acids on a microchip, some or all of the transcripts of a cell can be simultaneously quantified. Alternatively, arrays comprising genomic nucleic acid can also be used to determine the genotype of a newly engineered strain made by the methods of the invention. Polypeptide arrays" can also be used to simultaneously quantify a plurality of proteins. The present invention can be practiced with any known "array," also referred to as a "microarray" or "nucleic acid array" or "polypeptide array" or "antibody array" or "biochip," or variation thereof. Arrays are generically a plurality of "spots" or "target elements," each target element comprising a defined amount of one or more biological molecules, e.g., oligonucleotides, immobilized onto a defined area of a substrate surface for specific binding to a sample molecule, e.g., mRNA transcripts.

In practicing the methods of the invention, any known array and/or method of making and using arrays can be incorporated in whole or in part, or variations thereof, as described, for example, in U.S. Patent Nos. 6,277,628; 6,277,489; 6,261,776; 6,258,606; 6,054,270; 6,048,695; 6,045,996; 6,022,963; 6,013,440; 5,965,452; 5,959,098; 5,856,174; 5,830,645; 5,770,456; 5,632,957; 5,556,752; 5,143,854; 5,807,522; 5,800,992; 5,744,305; 5,700,637; 5,556,752; 5,434,049; see also, e.g., WO 99/51773; WO 99/09217; WO 97/46313; WO 96/17958; see also, e.g., Johnston (1998) Curr. Biol. 8:R171-R174; Schummer (1997) Biotechniques 23:1087-1092; Kern (1997) Biotechniques 23:120-124; Solinas-Toldo (1997) Genes, Chromosomes & Cancer 20:399-407; Bowtell (1999) Nature Genetics Supp. 21:25-32. See also published U.S. patent applications Nos. 20010018642; 20010019827; 20010016322; 20010014449; 20010014448; 20010012537; 20010008765.

Antibodies and Antibody-based screening methods

The invention provides isolated or recombinant antibodies that specifically bind to a xylanase of the invention. These antibodies can be used to isolate, identify or

quantify the xylanases of the invention or related polypeptides. These antibodies can be used to isolate other polypeptides within the scope the invention or other related xylanases. The antibodies can be designed to bind to an active site of a xylanase. Thus, the invention provides methods of inhibiting xylanases using the antibodies of the invention (see discussion above regarding applications for anti-xylanase compositions of the invention).

The invention provides fragments of the enzymes of the invention, including immunogenic fragments of a polypeptide of the invention. The invention provides compositions comprising a polypeptide or peptide of the invention and adjuvants or carriers and the like.

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The antibodies can be used in immunoprecipitation, staining, immunoaffinity columns, and the like. If desired, nucleic acid sequences encoding for specific antigens can be generated by immunization followed by isolation of polypeptide or nucleic acid, amplification or cloning and immobilization of polypeptide onto an array of the invention. Alternatively, the methods of the invention can be used to modify the structure of an antibody produced by a cell to be modified, e.g., an antibody's affinity can be increased or decreased. Furthermore, the ability to make or modify antibodies can be a phenotype engineered into a cell by the methods of the invention.

Methods of immunization, producing and isolating antibodies (polyclonal and monoclonal) are known to those of skill in the art and described in the scientific and patent literature, see, e.g., Coligan, CURRENT PROTOCOLS IN IMMUNOLOGY, Wiley/Greene, NY (1991); Stites (eds.) BASIC AND CLINICAL IMMUNOLOGY (7th ed.) Lange Medical Publications, Los Altos, CA ("Stites"); Goding, MONOCLONAL ANTIBODIES: PRINCIPLES AND PRACTICE (2d ed.) Academic Press, New York, NY (1986); Kohler (1975) Nature 256:495; Harlow (1988) ANTIBODIES, A LABORATORY MANUAL, Cold Spring Harbor Publications, New York. Antibodies also can be generated *in vitro*, e.g., using recombinant antibody binding site expressing phage display libraries, in addition to the traditional in vivo methods using animals. See, e.g., Hoogenboom (1997) Trends Biotechnol. 15:62-70; Katz (1997) Annu. Rev. Biophys. Biomol. Struct. 26:27-45.

The polypeptides of Group B amino acid sequences and sequences substantially identical thereto or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof, may also be used to generate antibodies which bind specifically to the polypeptides or fragments. The resulting antibodies may be used in immunoaffinity chromatography procedures to isolate or purify the polypeptide or to determine whether the polypeptide is present in a biological sample. In such procedures, a

protein preparation, such as an extract, or a biological sample is contacted with an antibody capable of specifically binding to one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof.

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In immunoaffinity procedures, the antibody is attached to a solid support, such as a bead or other column matrix. The protein preparation is placed in contact with the antibody under conditions in which the antibody specifically binds to one of the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragment thereof. After a wash to remove non-specifically bound proteins, the specifically bound polypeptides are eluted.

The ability of proteins in a biological sample to bind to the antibody may be determined using any of a variety of procedures familiar to those skilled in the art. For example, binding may be determined by labeling the antibody with a detectable label such as a fluorescent agent, an enzymatic label, or a radioisotope. Alternatively, binding of the antibody to the sample may be detected using a secondary antibody having such a detectable label thereon. Particular assays include ELISA assays, sandwich assays, radioimmunoassays and Western Blots.

Polyclonal antibodies generated against the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, for example, a nonhuman. The antibody so obtained will then bind the polypeptide itself. In this manner, even a sequence encoding only a fragment of the polypeptide can be used to generate antibodies which may bind to the whole native polypeptide. Such antibodies can then be used to isolate the polypeptide from cells expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, Nature, 256:495-497, 1975), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, Immunology Today 4:72, 1983) and the EBV-hybridoma technique (Cole, *et al.*, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to the polypeptides

of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof. Alternatively, transgenic mice may be used to express humanized antibodies to these polypeptides or fragments thereof.

Antibodies generated against the polypeptides of Group B amino acid sequences and sequences substantially identical thereto, or fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof may be used in screening for similar polypeptides from other organisms and samples. In such techniques, polypeptides from the organism are contacted with the antibody and those polypeptides which specifically bind the antibody are detected. Any of the procedures described above may be used to detect antibody binding. One such screening assay is described in "Methods for Measuring Cellulase Activities", *Methods in Enzymology*, Vol 160, pp. 87-116.

Kits

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The invention provides kits comprising the compositions, e.g., nucleic acids, expression cassettes, vectors, cells, transgenic seeds or plants or plant parts, polypeptides (e.g., xylanases) and/or antibodies of the invention. The kits also can contain instructional material teaching the methodologies and industrial uses of the invention, as described herein.

Whole cell engineering and measuring metabolic parameters

The methods of the invention provide whole cell evolution, or whole cell engineering, of a cell to develop a new cell strain having a new phenotype, e.g., a new or modified xylanase activity, by modifying the genetic composition of the cell. The genetic composition can be modified by addition to the cell of a nucleic acid of the invention, e.g., a coding sequence for an enzyme of the invention. See, e.g., WO0229032; WO0196551.

To detect the new phenotype, at least one metabolic parameter of a modified cell is monitored in the cell in a "real time" or "on-line" time frame. In one aspect, a plurality of cells, such as a cell culture, is monitored in "real time" or "on-line." In one aspect, a plurality of metabolic parameters is monitored in "real time" or "on-line." Metabolic parameters can be monitored using the xylanases of the invention.

Metabolic flux analysis (MFA) is based on a known biochemistry framework.

A linearly independent metabolic matrix is constructed based on the law of mass conservation and on the pseudo-steady state hypothesis (PSSH) on the intracellular metabolites. In practicing the methods of the invention, metabolic networks are established, including the:

- · identity of all pathway substrates, products and intermediary metabolites
- identity of all the chemical reactions interconverting the pathway metabolites, the stoichiometry of the pathway reactions,
 - identity of all the enzymes catalyzing the reactions, the enzyme reaction kinetics,
- the regulatory interactions between pathway components, e.g. allosteric interactions, enzyme-enzyme interactions etc,
- intracellular compartmentalization of enzymes or any other supramolecular organization of the enzymes, and,

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• the presence of any concentration gradients of metabolites, enzymes or effector molecules or diffusion barriers to their movement.

Once the metabolic network for a given strain is built, mathematic presentation by matrix notion can be introduced to estimate the intracellular metabolic fluxes if the on-line metabolome data is available. Metabolic phenotype relies on the changes of the whole metabolic network within a cell. Metabolic phenotype relies on the change of pathway utilization with respect to environmental conditions, genetic regulation, developmental state and the genotype, etc. In one aspect of the methods of the invention, after the on-line MFA calculation, the dynamic behavior of the cells, their phenotype and other properties are analyzed by investigating the pathway utilization. For example, if the glucose supply is increased and the oxygen decreased during the yeast fermentation, the utilization of respiratory pathways will be reduced and/or stopped, and the utilization of the fermentative pathways will dominate. Control of physiological state of cell cultures will become possible after the pathway analysis. The methods of the invention can help determine how to manipulate the fermentation by determining how to change the substrate supply, temperature, use of inducers, etc. to control the physiological state of cells to move along desirable direction. In practicing the methods of the invention, the MFA results can also be compared with transcriptome and proteome data to design experiments and protocols for metabolic engineering or gene shuffling, etc.

In practicing the methods of the invention, any modified or new phenotype can be conferred and detected, including new or improved characteristics in the cell. Any aspect of metabolism or growth can be monitored.

Monitoring expression of an mRNA transcript

In one aspect of the invention, the engineered phenotype comprises increasing or decreasing the expression of an mRNA transcript (e.g., a xylanase message) or generating

new (e.g., xylanase) transcripts in a cell. This increased or decreased expression can be traced by testing for the presence of a xylanase of the invention or by xylanase activity assays. mRNA transcripts, or messages, also can be detected and quantified by any method known in the art, including, e.g., Northern blots, quantitative amplification reactions, hybridization to arrays, and the like. Quantitative amplification reactions include, e.g., quantitative PCR, including, e.g., quantitative reverse transcription polymerase chain reaction, or RT-PCR; quantitative real time RT-PCR, or "real-time kinetic RT-PCR" (see, e.g., Kreuzer (2001) Br. J. Haematol. 114:313-318; Xia (2001) Transplantation 72:907-914).

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In one aspect of the invention, the engineered phenotype is generated by knocking out expression of a homologous gene. The gene's coding sequence or one or more transcriptional control elements can be knocked out, e.g., promoters or enhancers. Thus, the expression of a transcript can be completely ablated or only decreased.

In one aspect of the invention, the engineered phenotype comprises increasing the expression of a homologous gene. This can be effected by knocking out of a negative control element, including a transcriptional regulatory element acting in cis- or trans-, or, mutagenizing a positive control element. One or more, or, all the transcripts of a cell can be measured by hybridization of a sample comprising transcripts of the cell, or, nucleic acids representative of or complementary to transcripts of a cell, by hybridization to immobilized nucleic acids on an array.

Monitoring expression of a polypeptides, peptides and amino acids

In one aspect of the invention, the engineered phenotype comprises increasing or decreasing the expression of a polypeptide (e.g., a xylanase) or generating new polypeptides in a cell. This increased or decreased expression can be traced by determining the amount of xylanase present or by xylanase activity assays. Polypeptides, peptides and amino acids also can be detected and quantified by any method known in the art, including, e.g., nuclear magnetic resonance (NMR), spectrophotometry, radiography (protein radiolabeling), electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, various immunological methods, e.g. immunoprecipitation, immunodiffusion, immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immuno-fluorescent assays, gel electrophoresis (e.g., SDS-PAGE), staining with antibodies, fluorescent activated cell sorter (FACS), pyrolysis mass spectrometry, Fourier-Transform Infrared Spectrometry, Raman spectrometry, GC-MS, and LC-Electrospray and

cap-LC-tandem-electrospray mass spectrometries, and the like. Novel bioactivities can also be screened using methods, or variations thereof, described in U.S. Patent No. 6,057,103. Furthermore, as discussed below in detail, one or more, or, all the polypeptides of a cell can be measured using a protein array.

Industrial Applications 5

The xylanase enzymes of the invention can be highly selective catalysts. They can catalyze reactions with exquisite stereo-, regio- and chemo- selectivities that are unparalleled in conventional synthetic chemistry. Moreover, enzymes are remarkably versatile. The xylanase enzymes of the invention can be tailored to function in organic solvents, operate at extreme pHs (for example, high pHs and low pHs) extreme temperatures (for example, high temperatures and low temperatures), extreme salinity levels (for example, high salinity and low salinity) and catalyze reactions with compounds that are structurally unrelated to their natural, physiological substrates.

Detergent Compositions

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The invention provides detergent compositions comprising one or more polypeptides (e.g., xylanases) of the invention, and methods of making and using these compositions. The invention incorporates all methods of making and using detergent compositions, see, e.g., U.S. Patent No. 6,413,928; 6,399,561; 6,365,561; 6,380,147. The detergent compositions can be a one and two part aqueous composition, a non-aqueous liquid composition, a cast solid, a granular form, a particulate form, a compressed tablet, a gel and/or a paste and a slurry form. The xylanases of the invention can also be used as a detergent additive product in a solid or a liquid form. Such additive products are intended to supplement or boost the performance of conventional detergent compositions and can be added at any stage of the cleaning process.

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The actual active enzyme content depends upon the method of manufacture of a detergent composition and is not critical, assuming the detergent solution has the desired enzymatic activity. In one aspect, the amount of xylanase present in the final solution ranges from about 0.001 mg to 0.5 mg per gram of the detergent composition. The particular enzyme chosen for use in the process and products of this invention depends upon the conditions of final utility, including the physical product form, use pH, use temperature, and soil types to be degraded or altered. The enzyme can be chosen to provide optimum activity and stability for any given set of utility conditions. In one aspect, the xylanases of the present invention are active in the pH ranges of from about 4 to about 12 and in the temperature

range of from about 20°C to about 95°C. The detergents of the invention can comprise cationic, semi-polar nonionic or zwitterionic surfactants; or, mixtures thereof.

Xylanases of the invention can be formulated into powdered and liquid detergents having pH between 4.0 and 12.0 at levels of about 0.01 to about 5% (preferably 0.1% to 0.5%) by weight. These detergent compositions can also include other enzymes such as xylanases, cellulases, lipases or endoglycosidases, endo-beta.-1,4-glucanases, beta-glucanases, endo-beta-1,3(4)-glucanases, cutinases, peroxidases, laccases, amylases, glucoamylases, pectinases, reductases, oxidases, phenoloxidases, ligninases, pullulanases, arabinanases, hemicellulases, mannanases, xyloglucanases, xylanases, pectin acetyl esterases, rhamnogalacturonan acetyl esterases, polygalacturonases, rhamnogalacturonases, galactanases, pectin lyases, pectin methylesterases, cellobiohydrolases and/or transglutaminases. These detergent compositions can also include builders and stabilizers.

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The addition of xylanases of the invention to conventional cleaning compositions does not create any special use limitation. In other words, any temperature and pH suitable for the detergent is also suitable for the compositions of the invention as long as the enzyme is active at or tolerant of the pH and/or temperature of the intended use. In addition, the xylanases of the invention can be used in a cleaning composition without detergents, again either alone or in combination with builders and stabilizers.

The present invention provides cleaning compositions including detergent compositions for cleaning hard surfaces, detergent compositions for cleaning fabrics, dishwashing compositions, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning solutions.

In one aspect, the invention provides a method for washing an object comprising contacting the object with a polypeptide of the invention under conditions sufficient for washing. A xylanase of the invention may be included as a detergent additive. The detergent composition of the invention may, for example, be formulated as a hand or machine laundry detergent composition comprising a polypeptide of the invention. A laundry additive suitable for pre-treatment of stained fabrics can comprise a polypeptide of the invention. A fabric softener composition can comprise a xylanase of the invention. Alternatively, a xylanase of the invention can be formulated as a detergent composition for use in general household hard surface cleaning operations. In alternative aspects, detergent additives and detergent compositions of the invention may comprise one or more other enzymes such as a xylanase, a lipase, a cutinase, another xylanase, a carbohydrase, a cellulase, a pectinase, a mannanase, an arabinase, a galactanase, a xylanase, an oxidase, e.g.,

a lactase, and/or a peroxidase (see also, above). The properties of the enzyme(s) of the invention are chosen to be compatible with the selected detergent (i.e. pH-optimum, compatibility with other enzymatic and non-enzymatic ingredients, etc.) and the enzyme(s) is present in effective amounts. In one aspect, xylanase enzymes of the invention are used to remove malodorous materials from fabrics. Various detergent compositions and methods for making them that can be used in practicing the invention are described in, e.g., U.S. Patent Nos. 6,333,301; 6,329,333; 6,326,341; 6,297,038; 6,309,871; 6,204,232; 6,197,070; 5,856,164.

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When formulated as compositions suitable for use in a laundry machine washing method, the xylanases of the invention can comprise both a surfactant and a builder compound. They can additionally comprise one or more detergent components, e.g., organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil suspension and anti-redeposition agents and corrosion inhibitors. Laundry compositions of the invention can also contain softening agents, as additional detergent components. Such compositions containing carbohydrase can provide fabric cleaning, stain removal, whiteness maintenance, softening, color appearance, dye transfer inhibition and sanitization when formulated as laundry detergent compositions.

The density of the laundry detergent compositions of the invention can range from about 200 to 1500 g/liter, or, about 400 to 1200 g/liter, or, about 500 to 950 g/liter, or, 600 to 800 g/liter, of composition; this can be measured at about 20°C.

The "compact" form of laundry detergent compositions of the invention is best reflected by density and, in terms of composition, by the amount of inorganic filler salt. Inorganic filler salts are conventional ingredients of detergent compositions in powder form. In conventional detergent compositions, the filler salts are present in substantial amounts, typically 17% to 35% by weight of the total composition. In one aspect of the compact compositions, the filler salt is present in amounts not exceeding 15% of the total composition, or, not exceeding 10%, or, not exceeding 5% by weight of the composition. The inorganic filler salts can be selected from the alkali and alkaline-earth-metal salts of sulphates and chlorides, e.g., sodium sulphate.

Liquid detergent compositions of the invention can also be in a "concentrated form." In one aspect, the liquid detergent compositions can contain a lower amount of water, compared to conventional liquid detergents. In alternative aspects, the water content of the concentrated liquid detergent is less than 40%, or, less than 30%, or, less than 20% by weight

of the detergent composition. Detergent compounds of the invention can comprise formulations as described in WO 97/01629.

Xylanases of the invention can be useful in formulating various cleaning compositions. A number of known compounds are suitable surfactants including nonionic, anionic, cationic, or zwitterionic detergents, can be used, e.g., as disclosed in U.S. Patent Nos. 4,404,128; 4,261,868; 5,204,015. In addition, xylanases can be used, for example, in bar or liquid soap applications, dish care formulations, contact lens cleaning solutions or products, peptide hydrolysis, waste treatment, textile applications, as fusion-cleavage enzymes in protein production, and the like. Xylanases may provide enhanced performance in a detergent composition as compared to another detergent xylanase, that is, the enzyme group may increase cleaning of certain enzyme sensitive stains such as grass or blood, as determined by usual evaluation after a standard wash cycle. Xylanases can be formulated into known powdered and liquid detergents having pH between 6.5 and 12.0 at levels of about 0.01 to about 5% (for example, about 0.1% to 0.5%) by weight. These detergent cleaning compositions can also include other enzymes such as known xylanases, xylanases, amylases, cellulases, lipases or endoglycosidases, as well as builders and stabilizers.

In one aspect, the invention provides detergent compositions having xylanase activity (a xylanase of the invention) for use with fruit, vegetables and/or mud and clay compounds (see, for example, U.S. Pat. No. 5,786,316).

Treating fibers and textiles

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The invention provides methods of treating fibers and fabrics using one or more xylanases of the invention. The xylanases can be used in any fiber- or fabric-treating method, which are well known in the art, see, e.g., U.S. Patent No. 6,261,828; 6,077,316; 6,024,766; 6,021,536; 6,017,751; 5,980,581; US Patent Publication No. 20020142438 A1. For example, xylanases of the invention can be used in fiber and/or fabric desizing. In one aspect, the feel and appearance of a fabric is improved by a method comprising contacting the fabric with a xylanase of the invention in a solution. In one aspect, the fabric is treated with the solution under pressure. For example, xylanases of the invention can be used in the removal of stains.

The xylanases of the invention can be used to treat any cellulosic material, including fibers (e.g., fibers from cotton, hemp, flax or linen), sewn and unsewn fabrics, e.g., knits, wovens, denims, yarns, and toweling, made from cotton, cotton blends or natural or manmade cellulosics (e.g. originating from xylan-containing cellulose fibers such as from

wood pulp) or blends thereof. Examples of blends are blends of cotton or rayon/viscose with one or more companion material such as wool, synthetic fibers (e.g. polyamide fibers, acrylic fibers, polyester fibers, polyvinyl alcohol fibers, polyvinyl chloride fibers, polyvinylidene chloride fibers, polyurethane fibers, polyurea fibers, aramid fibers), and cellulose-containing fibers (e.g. rayon/viscose, ramie, hemp, flax/linen, jute, cellulose acetate fibers, lyocell).

The textile treating processes of the invention (using xylanases of the invention) can be used in conjunction with other textile treatments, e.g., scouring and bleaching. Scouring is the removal of non-cellulosic material from the cotton fiber, e.g., the cuticle (mainly consisting of waxes) and primary cell wall (mainly consisting of pectin, protein and xyloglucan). A proper wax removal is necessary for obtaining a high wettability. This is needed for dyeing. Removal of the primary cell walls by the processes of the invention improves wax removal and ensures a more even dyeing. Treating textiles with the processes of the invention can improve whiteness in the bleaching process. The main chemical used in scouring is sodium, hydroxide in high concentrations and at high temperatures. Bleaching comprises oxidizing the textile. Bleaching typically involves use of hydrogen peroxide as the oxidizing agent in order to obtain either a fully bleached (white) fabric or to ensure a clean shade of the dye.

The invention also provides alkaline xylanases (xylanases active under alkaline conditions). These have wide-ranging applications in textile processing, degumming of plant fibers (e.g., plant bast fibers), treatment of pectic wastewaters, paper-making, and coffee and tea fermentations. See, e.g., Hoondal (2002) Applied Microbiology and Biotechnology 59:409-418.

Treating foods and food processing

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The xylanases of the invention have numerous applications in food processing industry. For example, in one aspect, the xylanases of the invention are used to improve the extraction of oil from oil-rich plant material, e.g., oil-rich seeds, for example, soybean oil from soybeans, olive oil from olives, rapeseed oil from rapeseed and/or sunflower oil from sunflower seeds.

The xylanases of the invention can be used for separation of components of
plant cell materials. For example, xylanases of the invention can be used in the separation of
xylan-rich material (e.g., plant cells) into components. In one aspect, xylanases of the
invention can be used to separate xylan-rich or oil-rich crops into valuable protein and oil and
hull fractions. The separation process may be performed by use of methods known in the art.

The xylanases of the invention can be used in the preparation of fruit or vegetable juices, syrups, extracts and the like to increase yield. The xylanases of the invention can be used in the enzymatic treatment (e.g., hydrolysis of xylan-comprising plant materials) of various plant cell wall-derived materials or waste materials, e.g. from cereals, grains, wine or juice production, or agricultural residues such as vegetable hulls, bean hulls, sugar beet pulp, olive pulp, potato pulp, and the like. The xylanases of the invention can be used to modify the consistency and appearance of processed fruit or vegetables. The xylanases of the invention can be used to treat plant material to facilitate processing of plant material, including foods, facilitate purification or extraction of plant components. The xylanases of the invention can be used to improve feed value, decrease the water binding capacity, improve the degradability in waste water plants and/or improve the conversion of plant material to ensilage, and the like.

In one aspect, xylanases of the invention are used in baking applications, e.g., cookies and crackers, to hydrolyze arabinoxylans and create non-sticky doughs that are not difficult to machine and to reduce biscuit size. Use xylanases of the invention to hydrolyze arabinoxylans is used to prevent rapid rehydration of the baked product resulting in loss of crispiness and reduced shelf-life. In one aspect, xylanases of the invention are used as additives in dough processing. In one aspect, xylanases of the invention are used in dough conditioning, wherein in one aspect the xylanases possess high activity over a temperature range of about 25-35°C and at near neutral pH (7.0-7.5). In one aspect, dough conditioning enzymes can be inactivated at the extreme temperatures of baking (>500°F).

In one aspect, xylanases of the invention are used as additives in dough processing to perform optimally under dough pH and temperature conditions. In one aspect, an enzyme of the invention is used for dough conditioning. In one aspect, a xylanase of the invention possesses high activity over a temperature range of 25-35°C and at near neutral pH (7.0-7.5). In one aspect, the enzyme is inactivated at the extreme temperatures of baking, for example, >500°F.

Paper or pulp treatment

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The xylanases of the invention can be in paper or pulp treatment or paper deinking. For example, in one aspect, the invention provides a paper treatment process using a xylanase of the invention. In one aspect, the xylanase of the invention is applicable both in reduction of the need for a chemical bleaching agent, such as chlorine dioxide, and in high alkaline and high temperature environments. In one aspect, the xylanase of the invention is a

thermostable alkaline endoxylanase which can effect a greater than 25% reduction in the chlorine dioxide requirement of kraft pulp with a less than 0.5% pulp yield loss. In one aspect, boundary parameters are pH 10, 65-85°C and treatment time of less than 60 minutes at an enzyme loading of less than 0.001 wt%. A pool of xylanases may be tested for the ability to hydrolyze dye-labeled xylan at, for example, pH 10 and 60°C. The enzymes that test positive under these conditions may then be evaluated at, for example pH 10 and 70°C. Alternatively, enzymes may be tested at pH 8 and pH 10 at 70°C. In discovery of xylanases desirable in the pulp and paper industry libraries from high temperature or highly alkaline environments were targeted. Specifically, these libraries were screened for enzymes functioning at alkaline pH and a temperature of approximately 45°C. In another aspect, the xylanases of the invention are useful in the pulp and paper industry in degradation of a lignin hemicellulose linkage, in order to release the lignin.

Animal feeds and food or feed additives

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The invention provides methods for treating animal feeds and foods and food or feed additives using xylanases of the invention, animals including mammals (e.g., humans), birds, fish and the like. The invention provides animal feeds, foods, and additives comprising xylanases of the invention. In one aspect, treating animal feeds, foods and additives using xylanases of the invention can help in the availability of nutrients, e.g., starch, protein, and the like, in the animal feed or additive. By breaking down difficult to digest proteins or indirectly or directly unmasking starch (or other nutrients), the xylanase makes nutrients more accessible to other endogenous or exogenous enzymes. The xylanase can also simply cause the release of readily digestible and easily absorbed nutrients and sugars.

When added to animal feed, xylanases of the invention improve the *in vivo* break-down of plant cell wall material partly due to a reduction of the intestinal viscosity (see, e.g., Bedford et al., Proceedings of the 1st Symposium on Enzymes in Animal Nutrition, 1993, pp. 73-77), whereby a better utilization of the plant nutrients by the animal is achieved. Thus, by using xylanases of the invention in feeds the growth rate and/or feed conversion ratio (i.e. the weight of ingested feed relative to weight gain) of the animal is improved.

The animal feed additive of the invention may be a granulated enzyme product which may readily be-mixed with feed components. Alternatively, feed additives of the invention can form a component of a pre-mix. The granulated enzyme product of the invention may be coated or uncoated. The particle size of the enzyme granulates can be compatible with that of feed and pre-mix components. This provides a safe and convenient

mean of incorporating enzymes into feeds. Alternatively, the animal feed additive of the invention may be a stabilized liquid composition. This may be an aqueous or oil-based slurry. See, e.g., U.S. Patent No. 6,245,546.

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Xylanases of the present invention, in the modification of animal feed or a food, can process the food or feed either in vitro (by modifying components of the feed or food) or in vivo. Xylanases can be added to animal feed or food compositions containing high amounts of xylans, e.g. feed or food containing plant material from cereals, grains and the like. When added to the feed or food the xylanase significantly improves the in vivo break-down of xylan-containing material, e.g., plant cell walls, whereby a better utilization of the plant nutrients by the animal (e.g., human) is achieved. In one aspect, the growth rate and/or feed conversion ratio (i.e. the weight of ingested feed relative to weight gain) of the animal is improved. For example a partially or indigestible xylan-comprising protein is fully or partially degraded by a xylanase of the invention, e.g. in combination with another enzyme, e.g., beta-galactosidase, to peptides and galactose and/or galactooligomers. These enzyme digestion products are more digestible by the animal. Thus, xylanases of the invention can contribute to the available energy of the feed or food. Also, by contributing to the degradation of xylan-comprising proteins, a xylanase of the invention can improve the digestibility and uptake of carbohydrate and non-carbohydrate feed or food constituents such as protein, fat and minerals.

In another aspect, xylanase of the invention can be supplied by expressing the enzymes directly in transgenic feed crops (as, e.g., transgenic plants, seeds and the like), such as grains, cereals, corn, soy bean, rape seed, lupin and the like. As discussed above, the invention provides transgenic plants, plant parts and plant cells comprising a nucleic acid sequence encoding a polypeptide of the invention. In one aspect, the nucleic acid is expressed such that the xylanase of the invention is produced in recoverable quantities. The xylanase can be recovered from any plant or plant part. Alternatively, the plant or plant part containing the recombinant polypeptide can be used as such for improving the quality of a food or feed, e.g., improving nutritional value, palatability, and rheological properties, or to destroy an antinutritive factor.

In one aspect, the invention provides methods for removing oligosaccharides from feed prior to consumption by an animal subject using a xylanase of the invention. In this process a feed is formed having an increased metabolizable energy value. In addition to xylanases of the invention, galactosidases, cellulases and combinations thereof can be used. In one aspect, the enzyme is added in an amount equal to between about 0.1% and 1% by

weight of the feed material. In one aspect, the feed is a cereal, a wheat, a grain, a soybean (e.g., a ground soybean) material. See, e.g., U.S. Patent No. 6,399,123.

In another aspect, the invention provides methods for utilizing xylanase as a nutritional supplement in the diets of animals by preparing a nutritional supplement containing a recombinant xylanase enzyme comprising at least thirty contiguous amino acids of an amino acid of Group B amino acid sequences, and administering the nutritional supplement to an animal to increase the utilization of xylan contained in food ingested by the animal.

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In yet another aspect, the invention provides an edible pelletized enzyme delivery matrix and method of use for delivery of xylanase to an animal, for example as a nutritional supplement. The enzyme delivery matrix readily releases a xylanase enzyme, such as one having an amino acid sequence of group B amino acid sequences, or at least 30 contiguous amino acids thereof, in aqueous media, such as, for example, the digestive fluid of an animal. The invention enzyme delivery matrix is prepared from a granulate edible carrier selected from such components as grain germ that is spent of oil, hay, alfalfa, timothy, soy hull, sunflower seed meal, wheat midd, and the like, that readily disperse the recombinant enzyme contained therein into aqueous media. In use, the edible pelletized enzyme delivery matrix is administered to an animal to delivery of xylanase to the animal. Suitable grainbased substrates may comprise or be derived from any suitable edible grain, such as wheat, corn, soy, sorghum, alfalfa, barley, and the like. An exemplary grain-based substrate is a corn-based substrate. The substrate may be derived from any suitable part of the grain, but is preferably a grain germ approved for animal feed use, such as corn germ that is obtained in a wet or dry milling process. The grain germ preferably comprises spent germ, which is grain germ from which oil has been expelled, such as by pressing or hexane or other solvent extraction. Alternatively, the grain germ is expeller extracted, that is, the oil has been removed by pressing.

The enzyme delivery matrix of the invention is in the form of discrete plural particles, pellets or granules. By "granules" is meant particles that are compressed or compacted, such as by a pelletizing, extrusion, or similar compacting to remove water from the matrix. Such compression or compacting of the particles also promotes intraparticle cohesion of the particles. For example, the granules can be prepared by pelletizing the grain-based substrate in a pellet mill. The pellets prepared thereby are ground or crumbled to a granule size suitable for use as an adjuvant in animal feed. Since the matrix is itself approved for use in animal feed, it can be used as a diluent for delivery of enzymes in animal feed.

Preferably, the enzyme delivery matrix is in the form of granules having a granule size ranging from about 4 to about 400 mesh (USS); more preferably, about 8 to about 80 mesh; and most preferably about 14 to about 20 mesh. If the grain germ is spent via solvent extraction, use of a lubricity agent such as corn oil may be necessary in the pelletizer, but such a lubricity agent ordinarily is not necessary if the germ is expeller extracted. In other aspects of the invention, the matrix is prepared by other compacting or compressing processes such as, for example, by extrusion of the grain-based substrate through a die and grinding of the extrudate to a suitable granule size.

The enzyme delivery matrix may further include a polysaccharide component as a cohesiveness agent to enhance the cohesiveness of the matrix granules. The cohesiveness agent is believed to provide additional hydroxyl groups, which enhance the bonding between grain proteins within the matrix granule. It is further believed that the additional hydroxyl groups so function by enhancing the hydrogen bonding of proteins to starch and to other proteins. The cohesiveness agent may be present in any amount suitable to enhance the cohesiveness of the granules of the enzyme delivery matrix. Suitable cohesiveness agents include one or more of dextrins, maltodextrins, starches, such as corn starch, flours, cellulosics, hemicellulosics, and the like. For example, the percentage of grain germ and cohesiveness agent in the matrix (not including the enzyme) is 78% corn germ meal and 20% by weight of corn starch.

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Because the enzyme-releasing matrix of the invention is made from biodegradable materials, the matrix may be subject to spoilage, such as by molding. To prevent or inhibit such molding, the matrix may include a mold inhibitor, such as a propionate salt, which may be present in any amount sufficient to inhibit the molding of the enzyme-releasing matrix, thus providing a delivery matrix in a stable formulation that does not require refrigeration.

The xylanase enzyme contained in the invention enzyme delivery matrix and methods is preferably a thermostable xylanase, as described herein, so as to resist inactivation of the xylanase during manufacture where elevated temperatures and/or steam may be employed to prepare the palletized enzyme delivery matrix. During digestion of feed containing the invention enzyme delivery matrix, aqueous digestive fluids will cause release of the active enzyme. Other types of thermostable enzymes and nutritional supplements that are thermostable can also be incorporated in the delivery matrix for release under any type of aqueous conditions.

A coating can be applied to the invention enzyme matrix particles for many different purposes, such as to add a flavor or nutrition supplement to animal feed, to delay release of animal feed supplements and enzymes in gastric conditions, and the like. Or, the coating may be applied to achieve a functional goal, for example, whenever it is desirable to slow release of the enzyme from the matrix particles or to control the conditions under which the enzyme will be released. The composition of the coating material can be such that it is selectively broken down by an agent to which it is susceptible (such as heat, acid or base, enzymes or other chemicals). Alternatively, two or more coatings susceptible to different such breakdown agents may be consecutively applied to the matrix particles.

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The invention is also directed towards a process for preparing an enzymereleasing matrix. In accordance with the invention, the process comprises providing discrete plural particles of a grain-based substrate in a particle size suitable for use as an enzymereleasing matrix, wherein the particles comprise a xylanase enzyme encoded by an amino acid sequence of Group B amino acid sequences or at least 30 consecutive amino acids thereof. Preferably, the process includes compacting or compressing the particles of enzymereleasing matrix into granules, which most preferably is accomplished by pelletizing. The mold inhibitor and cohesiveness agent, when used, can be added at any suitable time, and preferably are mixed with the grain-based substrate in the desired proportions prior to pelletizing of the grain-based substrate. Moisture content in the pellet mill feed preferably is in the ranges set forth above with respect to the moisture content in the finished product, and preferably is about 14-15%. Preferably, moisture is added to the feedstock in the form of an aqueous preparation of the enzyme to bring the feedstock to this moisture content. The temperature in the pellet mill preferably is brought to about 82°C with steam. The pellet mill may be operated under any conditions that impart sufficient work to the feedstock to provide pellets. The pelleting process itself is a cost-effective process for removing water from the enzyme-containing composition.

In one aspect, the pellet mill is operated with a 1/8 in. by 2 in. die at 100 lb./min. pressure at 82°C. to provide pellets, which then are crumbled in a pellet mill crumbler to provide discrete plural particles having a particle size capable of passing through an 8 mesh screen but being retained on a 20 mesh screen.

The thermostable xylanases of the invention can be used in the pellets of the invention. They can have high optimum temperatures and high heat resistance such that an enzyme reaction at a temperature not hitherto carried out can be achieved. The gene encoding the xylanase according to the present invention (e.g. as set forth in any of the

sequences in Group A nucleic acid sequences) can be used in preparation of xylanases (e.g. using GSSMTM as described herein) having characteristics different from those of the xylanases of Group B amino acid sequences (in terms of optimum pH, optimum temperature, heat resistance, stability to solvents, specific activity, affinity to substrate, secretion ability, translation rate, transcription control and the like). Furthermore, a polynucleotide of Group A nucleic acid sequences may be employed for screening of variant xylanases prepared by the methods described herein to determine those having a desired activity, such as improved or modified thermostability or thermotolerance. For example, U.S. Patent No. 5,830,732, describes a screening assay for determining thermotolerance of a xylanase.

Waste treatment

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The xylanases of the invention can be used in a variety of other industrial applications, e.g., in waste treatment. For example, in one aspect, the invention provides a solid waste digestion process using xylanases of the invention. The methods can comprise reducing the mass and volume of substantially untreated solid waste. Solid waste can be treated with an enzymatic digestive process in the presence of an enzymatic solution (including xylanases of the invention) at a controlled temperature. This results in a reaction without appreciable bacterial fermentation from added microorganisms. The solid waste is converted into a liquefied waste and any residual solid waste. See e.g., U.S. Patent No. 5,709,796.

Oral care products

The invention provides oral care product comprising xylanases of the invention. Exemplary oral care products include toothpastes, dental creams, gels or tooth powders, odontics, mouth washes, pre- or post brushing rinse formulations, chewing gums, lozenges, or candy. See, e.g., U.S. Patent No. 6,264,925.

Brewing and fermenting

The invention provides methods of brewing (e.g., fermenting) beer comprising xylanases of the invention. In one exemplary process, starch-containing raw materials are disintegrated and processed to form a malt. A xylanase of the invention is used at any point in the fermentation process. For example, xylanases of the invention can be used in the processing of barley malt. The major raw material of beer brewing is barley malt. This can be a three stage process. First, the barley grain can be steeped to increase water content, e.g., to around about 40%. Second, the grain can be germinated by incubation at 15 to 25°C for 3 to 6 days when enzyme synthesis is stimulated under the control of gibberellins. In one

aspect, xylanases of the invention are added at this (or any other) stage of the process. Xylanases of the invention can be used in any beer or alcoholic beverage producing process, as described, e.g., in U.S. Patent No. 5,762,991; 5,536,650; 5,405,624; 5,021,246; 4,788,066.

In one aspect, an enzyme of the invention is used to improve filterability and wort viscosity and to obtain a more complete hydrolysis of endosperm components. Use of an enzyme of the invention would also increase extract yield. The process of brewing involves germination of the barley grain (malting) followed by the extraction and the breakdown of the stored carbohydrates to yield simple sugars that are used by yeast for alcoholic fermentation. Efficient breakdown of the carbohydrate reserves present in the barley endosperm and brewing adjuncts requires the activity of several different enzymes.

In one aspect, an enzyme of the invention has activity in slightly acidic pH (e.g., 5.5-6.0) in, e.g., the 40°C to 70°C temperature range; and, in one aspect, with inactivation at 95°C. Activity under such conditions would be optimal, but are not an essential requirement for efficacy. In one aspect, an enzyme of the invention has activity between 40-75° C, and pH 5.5-6.0; stable at 70° for at least 50 minutes, and, in one aspect, is inactivated at 96-100 °C. Enzymes of the invention can be used with other enzymes, e.g., beta-1,4-endoglucanases and amylases.

Medical and research applications

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Xylanases of the invention can be used as antimicrobial agents due to their bacteriolytic properties. Xylanases of the invention can be used to eliminating or protecting animals from salmonellae, as described in e.g., PCT Application Nos. WO0049890 and WO9903497.

Other industrial applications

Xylanases of the invention can be used, including Group B amino acid sequences are used in a wide variety of food, animal feed and beverage applications. New xylanases are discovered by screening existing libraries and DNA libraries constructed from diverse mesophilic and moderately thermophilic locations as well as from targeted sources including digestive flora, microorganisms in animal waste, soil bacteria and highly alkaline habitats. Biotrap and primary enrichment strategies using arabinoxylan substrates and/or non-soluble polysaccharide fractions of animal feed material are also useful.

Two screening formats (activity-based and sequence-based) are used in the discovery of novel xylanases. The activity-based approach is direct screening for xylanase activity in agar plates using a substrate such as AZO-xylan (Megazyme). Alternatively a

sequence-based approach may be used, which relies on bioinformatics and molecular biology to design probes for hybridization and biopanning. See, for example, U.S. Patents No. 6,054,267, 6,030,779, 6,368,798, 6,344,328. Hits from the screening are purified, sequenced, characterized (for example, determination of specificity, temperature and pH optima), analyzed using bioinformatics, subcloned and expressed for basic biochemical characterization. These methods may be used in screening for xylanases useful in a myriad of applications, including dough conditioning and as animal feed additive enzymes.

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In characterizing enzymes obtained from screening, the exemplary utility in dough processing and baking applications may be assessed. Characterization may include, for example, measurement of substrate specificity (xylan, arabinoxylan, CMC, BBG), temperature and pH stability and specific activity. A commercial enzyme may be used as a benchmark. In one aspect, the enzymes of the invention have significant activity at pH \geq 7 and 25-35° C, are inactive on insoluble xylan, are stable and active in 50-67% sucrose.

In another aspect, utility as feed additives may be assessed from characterization of candidate enzymes. Characterization may include, for example, measurement of substrate specificity (xylan, arabinoxylan, CMC, BβG), temperature and pH stability, specific activity and gastric stability. In one aspect the feed is designed for a monogastric animal and in another aspect the feed is designed for a ruminant animal. In one aspect, the enzymes of the invention have significant activity at pH 2-4 and 35-40°C, a half-life greater than 30 minutes in gastric fluid, formulation (in buffer or cells) half-life greater than 5 minutes at 85°C and are used as a monogastric animal feed additive. In another aspect, the enzymes of the invention have one or more of the following characteristics: significant activity at pH 6.5-7.0 and 35-40°C, a half-life greater than 30 minutes in rumen fluid, formulation stability as stable as dry powder and are used as a ruminant animal feed additive.

Enzymes are reactive toward a wide range of natural and unnatural substrates, thus enabling the modification of virtually any organic lead compound. Moreover, unlike traditional chemical catalysts, enzymes are highly enantio- and regio-selective. The high degree of functional group specificity exhibited by enzymes enables one to keep track of each reaction in a synthetic sequence leading to a new active compound. Enzymes are also capable of catalyzing many diverse reactions unrelated to their physiological function in nature. For example, peroxidases catalyze the oxidation of phenols by hydrogen peroxide. Peroxidases can also catalyze hydroxylation reactions that are not related to the native function of the

enzyme. Other examples are xylanases which catalyze the breakdown of polypeptides. In organic solution some xylanases can also acylate sugars, a function unrelated to the native function of these enzymes.

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The present invention exploits the unique catalytic properties of enzymes. Whereas the use of biocatalysts (i.e., purified or crude enzymes, non-living or living cells) in chemical transformations normally requires the identification of a particular biocatalyst that reacts with a specific starting compound, the present invention uses selected biocatalysts and reaction conditions that are specific for functional groups that are present in many starting compounds. Each biocatalyst is specific for one functional group, or several related functional groups and can react with many starting compounds containing this functional group. The biocatalytic reactions produce a population of derivatives from a single starting compound. These derivatives can be subjected to another round of biocatalytic reactions to produce a second population of derivative compounds. Thousands of variations of the original compound can be produced with each iteration of biocatalytic derivatization.

Enzymes react at specific sites of a starting compound without affecting the rest of the molecule, a process which is very difficult to achieve using traditional chemical methods. This high degree of biocatalytic specificity provides the means to identify a single active compound within the library. The library is characterized by the series of biocatalytic reactions used to produce it, a so-called "biosynthetic history". Screening the library for biological activities and tracing the biosynthetic history identifies the specific reaction sequence producing the active compound. The reaction sequence is repeated and the structure of the synthesized compound determined. This mode of identification, unlike other synthesis and screening approaches, does not require immobilization technologies and compounds can be synthesized and tested free in solution using virtually any type of screening assay. It is important to note, that the high degree of specificity of enzyme reactions on functional groups allows for the "tracking" of specific enzymatic reactions that make up the biocatalytically produced library.

Many of the procedural steps are performed using robotic automation enabling the execution of many thousands of biocatalytic reactions and screening assays per day as well as ensuring a high level of accuracy and reproducibility. As a result, a library of derivative compounds can be produced in a matter of weeks which would take years to produce using current chemical methods. (For further teachings on modification of molecules, including small molecules, see PCT/US94/09174).

The invention will be further described with reference to the following examples; however, it is to be understood that the invention is not limited to such examples.

EXAMPLES

5 <u>EXAMPLE 1: PLATE BASED ENDOGLYCOSIDASE ENZYME DISCOVERY:</u> EXPRESSION SCREENING

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Titer determination of Lambda Library: Add 1.0 μL of Lambda Zap Express amplified library stock to 600μL E. coli MRF' cells (OD₆₀₀=1.0). Dilute MRF' stock with 10mM MgSO₄. Incubate mixture at 37°C for 15 minutes, then transfer suspension to 5-6mL of NZY top agar at 50 °C and gently mix. Immediately pour agar solution onto large (150mm) NZY media plate and allow top agar to solidify completely (approximately 30 minutes). Invert the plate. Incubate the plate at 39°C for 8-12 hours. (The number of plaques is approximated. Phage titer determined to give 50,000 pfu/plate. Dilute an aliquot of Library phage with SM buffer if needed.)

Substrate screening: Add Lambda Zap Express (50,000 pfu) from amplified library to 600μL of *E. coli* MRF' cells (OD₆₀₀=1.0) and incubate at 37°C for 15 minutes. While phage/cell suspension is incubating, add 1.0mL of desired polysaccharide dye-labeled substrate (usually 1-2% w/v) to 5.0mL NZY top agar at 50°C and mix thoroughly. (Solution kept at 50°C until needed.) Transfer the cell suspension to substrate/top agar solution and gently mix. Immediately pour solution onto large (150mm) NZY media plate. Allow top agar to solidify completely (approximately 30 minutes), then invert plate. Incubate plate at 39°C for 8-12 hours. Observe plate for clearing zones (halos) around plaques. Core plaques with halos out of agar and transfer to a sterile micro tube. (A large bore 200μL pipette tip works well to remove (core) the agar plug containing the desired plaque.) Resuspend phage in 500μL SM buffer. Add 20μL chloroform to inhibit any further cell growth.

Isolation of pure clones: Add 5μL of resuspended phage suspension to 500μL of *E. coli* MRF' cells (OD₆₀₀=1.0). Incubate at 37°C for 15 minutes. While phage/cell suspension is incubating, add 600μL of desired polysaccharide dye-labeled substrate (usually 1-2% w/v) to 3.0mL NZY top agar at 50°C and mix thoroughly. (Solution kept at 50°C until needed.) Transfer cell suspension to substrate/top agar solution and gently mix. Immediately pour solution onto small (90mm) NZY media plate and allow top agar to solidify completely

(approximately 30 minutes), then invert plate. Incubate plate at 39°C for 8-12 hours. Plate observed for a clearing zone (halo) around a single plaque (pure clone). (If a single plaque cannot be isolated, adjust titer and replate phage suspension.) Phage are resuspended in 500µL SM buffer and 20µL Chloroform is added to inhibit any further cell growth.

- Excision of pure clone: Allow pure phage suspension to incubate at room temperature for 2 to 3 hours or overnight at 4°C. Add 100 µL of pure phage suspension to 200 µL E. coli MRF' cells (OD₆₀₀=1.0). Add 1.0 μ L of ExAssist helper phage (>1 x 10⁶ pfu/mL; Stratagene). Incubate suspension at 37°C for 15 minutes. Add 3.0 mL of 2 x YT media to cell suspension. Incubate at 37°C for 2-2.5 hours while shaking. Transfer tube to 70°C for 20 minutes.
- Transfer 50-100 μL of phagemid suspension to a micro tube containing 200μL of E. coli Exp 10 505 cells (OD600=1.0). Incubate suspension at 37°C for 45 minutes. Plate 100 μL of cell suspension on LB_{kan 50} media (LB media with Kanamycin 50µg/mL). Incubate plate at 37°C for 8-12 hours. Observe plate for colonies. Any colonies that grow contain the pure phagemid. Pick a colony and grow a small (3-10mL) liquid culture for 8-12 hours. Culture media is liquid LB kan 50. 15
 - Activity verification: Transfer 1.0mL of liquid culture to a sterile micro tube. Centrifuge at 13200 rpm (16000 g's) for 1 minute. Discard supernatant and add 200µL of phosphate buffer pH 6.2. Sonicate for 5 to 10 seconds on ice using a micro tip. Add 200 μL of appropriate substrate, mix gently and incubate at 37 °C for 1.5-2 hours. A negative control should also be run that contains only buffer and substrate. Add 1.0mL absolute ethanol (200 proof) to suspension and mixed. Centrifuge at 13200 rpm for 10 minutes. Observe supernatant for color. Amount of coloration may vary, but any tubes with more coloration than control is considered positive for activity. A spectrophotometer can be used for this step if so desired or needed. (For Azo-xylan, Megazyme, read at 590nm).

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RFLP of pure clones from same Libraries: Transfer 1.0mL of liquid culture to a sterile micro 25 tube. Centrifuge at 13200 rpm (16000 g's) for 1 minute. Follow QIAprep spin mini kit (Qiagen) protocol for plasmid isolation and use 40 µL holy water as the elution buffer. Transfer 10 μL plasmid DNA to a sterile micro tube. Add 1.5μL Buffer 3 (New England Biolabs), 1.5µL 100X BSA solution (New England Biolabs) and 2.0µL holy water. To this add 1.0µL Not 1 and 1.0µL Pst 1 restriction endonucleases (New England Biolabs). 30

digested sample on a 1.0% agarose gel for 1-1.5 hours at 120 volts. View the gel with a gel imager. Perform sequence analysis on all clones with a different digest pattern.

Table 6 describes various properties of exemplary enzymes of the invention.

Table 6

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lone in the !	Ŧ. 14	Take!	ml lo -4*	Cignificant	p.1	Δ.4	Notes
SEQ ID NO.	Topt*	Tstab T	pHopt*	Significant activities	pΙ	Mw	ivotes
							نــــــــــــــــــــــــــــــــــــــ
151, 152	50°C	<1 min at 65°C	5.5-9.0	AZO-xylan	5.7	40.2	
155, 156	50°C	<1 min at 65°C	5.5-8.0	AZO-xylan	8.8	62.7	
169, 170	50°C	> 1 min at 65°C; < 1 min at 85°C	7.0	AZO-xylan	8.7	36.7	٠
195, 196	50°C	>1 min at 65°C < 10 min, < 1 min 85°C	5.5	AZO-xylan	8.5	36.7	
215, 216	85°C	<3 min at 85°C	5.5-8.0	AZO-xylan	8.6	34.8	
47, 48	50°C	< 0.5 min at 65°C; < 1 min at 85°C	7.0-8.0	AZO-xylan	6.2	40.3	
191, 192	385°C	> 30 sec at 85°C	5.5	AZO-xylan	7.8	34.6	
247, 248	50°C	< 1 min at 65°C	8.0	AZO-xylan	9.4	43.5	
7, 8	50°C	> 1 min 85°C < 5 min	5.5	AZO-xylan	4.5	55.3	
221, 222	50-65°C	<1 min at 75°C	5.5	AZO-xylan	8.3	34.6	
163, 164	65°C	<1 min at 65°C	7.0	AZO-xylan	6.3	36.0	
19, 20	37°C	<5 min at 50°C	7.0 - 8.0	AZO-xylan	9.2	41.5	
87, 88	37 - 50°C	< 1 min at 85°C	8.0	AZO-xylan	5.2	36.7	
81, 82	50°C	< 1 min at 65°C	7.0 - 9.0	AZO-xylan	5.3	38.8	
91, 92	50°C	< 1 min at 65°C	7 - 8	AZO-xylan, AZO- CMC	5.4	39.0	
61, 62	37°C	<5 min at 50°C	7.0 - 9.0	AZO-xylan, AZO- CMC	5.4	40	
159, 160	85°C	< 30 sec at 85°C	5.5	AZO-xylan	8.3	34.5	
233, 234	50°C	> 30 sec < 1 min at 65°c; < 1 min at 85°C	7.0	AZO-xylan	8.5	35.1	
203, 204	50 - 65°C	> 1 min at 65°C < 5 min, < 1 min 85°C	5.5	AZO-xylan	9.5	21.7	
181, 182	385°C	> 1 min at 85°C	5.5-8.0	AZO-xylan	8.8	35.5	
227, 228	65°C	>1 min at 85°C < 5 min	5.5 - 7.0	AZO-xylan	7.8	25.8	
45, 46	³45°C	³ 5 min 45°C, <0.5 min 55°C	> 5.5	AZO-xylan	6.7	40.4	***
231, 232	65°C	>10 min at 50°C	5.5 - 7.0	AZO-xylan	8.4	31.4	
129, 130	65°C	<1min at 75°C	5.5	AZO-xylan	5.1	116	
93, 94	50°C	< 1 min at 60°C	8.0 - 9.0	AZO-xylan	5.3	39.1	
189, 190	65°C	<1 min at 65°C	5.5	AZO-xylan	9.2	20.3	***
49, 50	70°C	<20 min 70°C	>5	AZO-xylan	5.7	38.9	
85, 86	50°C	>5 min at 85°C	5.5 - 7.0	AZO-xylan	6.1	48.4	
99, 100	50°C	<1 min at 75°C	5.5 - 8.0	AZO-xylan	10.8	36.6	
123, 124	385°C	<30 sec 100 °C	5.5-7.0	AZO-xylan	6.1	44.1	
249, 250	45°C	>1 min 75°C < 10 min	5.5	AZO-xylan	5.3	93	
167, 168	85°C	< 5 min 85°C	5.5	AZO-xylan	9.5	21.7	
207, 208	75°C	< 5 min 65 °C	5.5	AZO-xylan	9.1	20.4	
251, 252	65-75°C	< 1 min 85 °C	5.5	AZO-xylan	8.8	20.4	****
11, 12	<90°C	<40 min 70°C	>6	AZO-xylan	6.8	43.9	
177, 178	65°C	< 1 min at 75°C	5.5	AZO-xylan	8.7	44.6	
9, 10	50°C	<1min at 65°C	5.5 - 7.0	AZO-xylan	4.9	46.1	
43, 44	37°C	unstable	5.5-7.0	AZO-xylan	4.9	39.1	
113, 114	65 - 75°C	< 1 min at 75°C	5.5 - 8.0	AZO-xylan	5	41.2	

SEQ ID NO.	Topt*	Tstab "	pHopt*	Significant activities	pl	Mw	Notes
75, 76	50°C	< 1 min 85°C	7.0 - 9.0	AZO-xylan	4.7	39.4	
111, 112	37°C	>10 min 50°C	7-8	AZO-xylan	5.6	41.0	
117, 118	37°C	unstable	7-8	AZO-xylan	9.1	53.3	
115, 116	-	-	-	AZO-xylan	8.9	50.8	
125, 126	37°C	-	8.0	AZO-xylan	5.3	41.1	
137, 138	50°C	< 30 sec at 65°C	5.5	AZO-xylan	5.7	38.5	
69, 70	385°C	< 5 min at 85°C	5.5-9.0	AZO-xylan	6.4	58.0	
205, 206	50°C	<1min at 65°C	5.5 - 8	AZO-xylan	4.3	35.1	
211, 212	50°C	<1min at 65°C	5.5	AZO-xylan	4.4	35.4	
197, 198	65°C	<1 min at 65°C	5.5	AZO-xylan	8.8	20.1	
31, 32	37°C	unstable	7.0	AZO-xylan	5.1	54.4	
13, 14	50°C	<1 min at 65°C	7	AZO-xylan	5.5	40.0	
65, 66	50°C	< 1 min at 65°C	5.5	AZO-xylan, AZO- CMC	4.8	55.5	
257, 258	37°C	unstable	5.5	AZO-xylan, AZO- barley β-glucan, AZO-CMC	5.3	100.8	
57, 58	50°C	<1min at 65°C	7.0	AZO-xylan	4.8	56.7	
185, 186	50-75°C	< 1 min at 80°C	5.5	AZO-xylan	8.8	23.2	
243, 244	75°C	>0.5 min @ 85°C	5.5	AZO-xylan	8.8	44.4	
77, 78	50°C	< 5 min at 65°C, < 1 min 85°C	5.5	AZO-xylan	5.3	44.5	
229, 230	37°C	³30 min 55°C, < 5 mln 75°C	5.5	AZO-xylan	8.7	20.6	*****
109, 110	65°C	>0.5 min @ 75°C	5.5	AZO-xylan	4.9	45.2	
193, 194	65°C	< 1 min at 75°C	5.5	AZO-xylan	5.4	29.1	
173, 174	65°C	< 1 min at 80°C	7.0	AZO-xylan	7.6	51.6	
59, 60	37°C	<1min at 65°C	7.0	AZO-xylan	6.6	42.5	
101, 102	50°C	>0.5 min @ 65°C	7.0	AZO-xylan	8.7	41.1	
55, 56	37°C	> 5 min at 50°C; < 1 min at 85°C	7.0	AZO-xylan	6.5	41.8	
15, 16	50°C	< 1 min at 65°C	7.0	AZO-xylan	6.4	40.2	
131, 132	_	•	-	AZO-xylan	5.6	42.1	
145, 146	65-85°C	< 1 min at 85°C	5.5	AZO-xylan	5.2	43.7	
219, 220	-	-	5.5	AZO-xylan	6.6	34.5	
253, 254	65°C	> .5 min at 85°C	5.5 - 7	AZO-xylan	7.8	34.6	
255, 256	65°C	> 1 min 65°C <3 min	5.5-7.0	AZO-xylan	8.3	35.0	

^{*} pH or temperature optima determined by initial rates using AZO-AZO-xylan as a substrate

EXAMPLE 2: GSSM™ SCREEN FOR THERMAL TOLERANT MUTANTS

The following example describes an exemplary method for screening for

10 thermally tolerant enzymes.

Master Plates: Prepare plates for a colony picker by labeling 96 well plates and aliquoting 200 µL LB Amp100 into each well. (~20ml needed per 96 well plate). After the plates are

^{**} thermal stability, time that enzyme retained significant activity (approx. > 50 %)

^{***} Dough conditioning

^{****} GSSMTM parent for thermal tolerance evolution for animal feed applications

^{*****} N35D mutation made to increase low pH activity- based on public knowledge- mutant enzyme's relative activity at pH 4 significantly increased

^{*****} Dough conditioning

returned from the picker, remove media from row 6 from plate A. Replace with an inoculation of SEQ ID NO: 189. Place in a humidified 37°C incubator overnight.

Assay Plates: Pin tool cultures into a fresh 96 well plate (200 µL /well LB Amp100).

Remove plastic cover and replace with Gas Permeable Seal. Place in a humidified incubator overnight. Remove the seal and replace plastic lid. Spin cultures down in tabletop centrifuge at 3000 rpm for 10 min. Remove supernatant by inversion onto a paper towel. Aliquot 45 µL Cit-Phos-KCl buffer pH 6 into each well. Replace the plastic lid with an aluminum plate seal. Use a roller to get a good seal. Resuspend cells in a plate shaker at level 6-7 for ~30 seconds.

Place the 96 well plate in 80°C incubator for 20 minutes. Do not stack. Thereafter, immediately remove plates to ice water to cool for a few minutes. Remove the aluminum seal and replace with a plastic lid. Add 30 μ L of 2 % Azo-xylan. Mix as before on the plate shaker. Incubate 37°C in a humidified incubator overnight.

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Add 200 μ L ethanol to each well and pipette up and down a couple of times to mix. As an alternative to changing tips each time, rinse in an ethanol wash and dry by expelling into a paper towel. Spin the plates at 3000 rpm for 10 minutes. Remove 100 μ L of supernatant to a fresh 96 well plate. Read the OD₅₉₀.

EXAMPLE 3: GSSM™ ASSAY FOR HIT VERIFICATION OF THERMAL TOLERANT MUTANTS

The following example describes an exemplary method for assaying for thermally tolerant enzymes.

Pin tool or pick clones into duplicate 96 well plates (200ul /well LB Amp100). Remove the plastic cover and replace with a Gas Permeable Seal. Place in a humidified incubator overnight. Remove the Seal and replace with a plastic lid. Pintool the clones to solid agar. Spin cultures down in tabletop centrifuge at 3000 rpm for 10 min. Remove the supernatant by inversion onto a paper towel. Aliquot 25 µl BPER/Lysozyme/DNase solution (see below) into each well. Resuspend cells in a plate shaker on level 6-7 for ~30 seconds.

Incubate the plate on ice for 15 minutes. Add 20 μ L of Cit-Phos-KCl buffer pH 6 into each well. Replace the plastic lid with an aluminum plate seal. Use a roller to get a good seal. Mix on a plate shaker at level 6-7 for ~30 seconds.

Place one 96 well plate in an 80°C incubator for 20 minutes and the other at 37°C. Do not stack. Immediately remove the plates to watery ice to cool for a few minutes (use a large plastic tray if needed). Remove the aluminum seal. Add 30 µl of 2% Azo-xylan.

Seal with a plastic gas permeable seal. Mix as before on the plate shaker. Incubate a set of 37°C and 80°C plates in humidified incubator at 37°C for 2 hours and another set for 4 hours.

After incubation, let the plate sit for \sim 5 minutes at room temperature. Add 200 μ L ethanol to each well and pipette up and down a couple of times to mix. Instead of changing tips each time, rinse in an ethanol wash and dry by expelling into a paper towel. But, use a new set of tips for each clone. Spin plates at 3000 rpm 10 minutes. Remove 100 μ L of supernatant to a fresh 96 well plate. Read OD₅₉₀.

BPER/Lysozyme/DNase solution (4.74 mL total): 4.5 mL BPR

200 μL 10 mg/mL Lysozyme (made fresh in pH 6 Cit-phos-buffer)
 40 μL 5 mg/mL DNase I (made fresh in pH 6 Cit-phos buffer

EXAMPLE 4: Xylanase assay with wheat arabinoxylan as substrate

The following example describes an exemplary xylanase assay that can be used, for example, to determine is an enzyme is within the scope of the invention.

SEQ ID NOS: 11, 12, 69, 70, 77, 78, 113, 114, 149, 150, 159, 160, 163, 164, 167, 168, 181, 182, 197, and 198 were subjected to an assay at pH 8 (Na-phosphate buffer) and 70°C using wheat arabinoxylan as a substrate. The enzymes were characterized as set forth in Table 7.

Table 7

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SEQ ID NOS:	Protein Concentration (mg/ml)	volume of lysate added to each vial	#of vials	Units/ml*	protein (mg/mL)	U/mg
11, 12	42	0.5	10	163	22.0	7.4
113, 114	37	0.6	10	66	22.0	3.0
163, 164	35	0.6	10	25	22.0	1.1
197, 198	23	1.0	10	31	22.0	1.4
167, 168	10	2.2	10	228	22.0	10.4
77, 78	47	0.5	10	29	22.0	1.3
69, 70	18	1.3	10	36	22.0	1.7
181, 182	28	0.8	10	24	22.0	1.1
159, 160	25	0.9	10	43	22.0	2.0
149, 150	42	0.5	10	24	22.0	1.1

^{*}Based on addition of 1 mL of water to each sample.

EXAMPLE 5: Generation of an exemplary xylanase of the invention

The following example describes the generation of an exemplary xylanase of the invention using gene site-saturation mutagenesis (GSSMTM) technology, designated the

Units are umoles xylose released per minute based on a reducing sugar assay.

"9x" variant or mutant (the nucleic acid as set forth in SEQ ID NO:377, the polypeptide sequence as set forth in SEQ ID NO:378).

GSSM™ was used to create a comprehensive library of point mutations in the exemplary SEQ ID NO:190, "wild-type" xylanase (encoded by SEQ ID NO:189). The xylanase thermotolerance screen described above identified nine single site amino acid mutants (Figure 6A) (D8F, Q11H, N12L, G17I, G60H, P64V, S65V, G68A & S79P) that had improved thermal tolerance relative to the wild type enzyme (as measured following a heat challenge at 80°C for 20 minutes). Wild-type enzyme and all nine single site amino acid mutants were produced in *E. coli* and purified utilizing an N-terminal hexahistidine tag. There was no noticeable difference in activity due to the tag.

Figure 6 illustrates the nine single site amino acid mutants of "variant 9x", or, as set forth in SEQ ID NO:378 (encoded by SEQ ID NO:377), as generated by Gene Site Saturation Mutagenesis (GSSMTM) of the exemplary SEQ ID NO:190 "wild-type" enzyme (encoded by SEQ ID NO:189). Figure 6A is a schematic diagram illustrating position, numbering and the amino acid change for the thermal tolerant point mutants of the "wild-type" gene (SEQ ID NO:190, encoded by SEQ ID NO:189). A library of all 64 codons was generated for every amino acid position in the gene (~13,000 mutants) and screened for mutations that increased thermal tolerance. The "9X" variant was generated by combining all 9 single-site mutants into one enzyme. The corresponding melting temperature transition midpoint (Tm) determined by DSC for each mutant enzyme and the "9X" (SEQ ID NO:378) variant is shown on the right. Figure 6B illustrates the unfolding of the "wild-type" (SEQ ID NO:190) and "9X" (SEQ ID NO:378) "variant/mutant" enzymes was monitored by DSC at a scan rate of 1°C/min. Baseline subtracted DSC data were normalized for protein concentration.

25 Xylanase activity assays

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Enzymatic activities were determined using 400 ∝L of 2% Azo-xylan as substrate in 550 ∝L of CP (citrate-phosphate) buffer, pH 6.0 at the indicated temperatures. Activity measurements as a function of pH were determined using 50 mM Britton and Robinson buffer solutions (pH 3.0, 5.0, 6.0, 7.0, 8.0 and 9.0) prepared by mixing solutions of 0.1 M phosphoric acid solution, 0.1 M boric acid and 0.1 M acetic acid followed by pH adjustment with 1 M sodium hydroxide. Reactions were initiated by adding 50 ∝L of 0.1 mg/ml of purified enzyme. Time points were taken from 0 to 15 minutes where 50 ∝L of reaction mixture was added to 200 ∝L of precipitation solution (100% ethanol). When all

time points had been taken, samples were mixed, incubated for 10 minutes and centrifuged at 3000 g for 10 minutes at 4°C. Supernatant (150 ∞L) was aliquoted into a fresh 96 well plate and absorbance was measured at 590 nm. As values were plotted against time and the initial rate was determined from the slope of the line.

5 Differential Scanning Calorimetry (DSC).

Calorimetry was performed using a Model 6100 Nano II DSC apparatus (Calorimetry Sciences Corporation, American Fork, UT) using the DSCRun software package for data acquisition, CpCalc for analysis, CpConvert for conversion into molar heat capacity from microwatts and CpDeconvolute for deconvolution. Analysis was carried out with 1mg/ml recombinant protein in 20 mM potassium phosphate (pH 7.0) and 100 mM KCl at a scan rate of 1oC/min. A constant pressure of 5 atm was maintained during all DSC experiments to prevent possible degassing of the solution on heating. The instrumental baseline was recorded routinely before the experiments with both cells filled with buffer. Reversibility of the thermally induced transitions was tested by reheating the solution in the calorimeter cell immediately after cooling the first run.

Thermal tolerance determination.

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All enzymes were analyzed for thermal tolerance at 80°C in 20 mM potassium phosphate (pH 7.0) and 100 mM KCl. The enzymes were heated at 80°C for 0, 5, 10 or 30 minutes in thin-walled tubes and were cooled on ice. Residual activities were determined with Azo-xylan as substrate using the assay described above for activity measurement.

Polysaccharide Fingerprinting.

Polysaccharide fingerprints were determined by polysaccharide analysis using carbohydrate gel electrophoresis (PACE). Beechwood xylan (0.1 mg/mL, $100 \, \infty L$, Sigma, Poole, Dorset, UK) or xylooligosaccharides (1 mM, $20 \, \infty L$, Megazyme, Wicklow, Ireland) were treated with enzyme $(1-3 \, \infty g)$ in a total volume of $250 \, \infty L$ for 16 hours. The reaction was buffered in 0.1 M ammonium acetate pH 5.5. Controls without substrates or enzymes were performed under the same conditions to identify any unspecific compounds in the enzymes, polysaccharides/oligosaccharides or labeling reagents. The reactions were stopped by boiling for 20 min. Assays were independently performed at least 2 times for each condition. Derivatization using ANTS (8-aminonaphthalene-1,3,6-trisulfonic acid, Molecular Probes, Leiden, The Netherlands), electrophoresis and imaging were carried out as described (Goubet, F., Jackson, P., Deery, M. and Dupree, P. (2002) Anal. Biochem. 300, 53-68).

Fitness Calculation.

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The fitness (F_n), for a given enzyme variant, n, was calculated by equally weighting increase in denaturation temperature transition midpoint (T_m) and increase (or decrease) in enzymatic activity relative to the largest difference in each parameter across all variants: $F_n = F_{Tn} + F_{Vn}$, where $F_{Tn} = T_m$ fitness factor of the variant and $F_{Vn} =$ activity fitness factor of the variant. The fitness factors for each (T_m and activity) are relative to the largest difference in T_m or rate across all of the variants. $F_{Tn} = (T_m - T_{mL}) / (T_{mH} - T_{mL})$ where T_{mn} is the T_m for the given variant, n, and T_{mL} is the lowest T_m across all variants and T_{mH} the highest T_m across all variants and $F_{Vn} = (V_n - V_L) / (V_H - V_L)$ where V_n is the relative rate for the given variant, n, and V_L is the lowest rate across all variants and V_H the highest rate across all variants.

Evolution by the GSSM[™] method.

GSSMTM technology was used to create a comprehensive library of point mutations in the exemplary xylanase of the invention SEQ ID NO:190 (encoded by SEQ ID NO:189); including the exemplary xylanase of the invention SEQ ID NO:378 (encoded by SEQ ID NO:377). The xylanase thermotolerance screen described above identified nine single site amino acid mutants (Figure 6A), D8F, Q11H, N12L, G17I, G60H, P64V, S65V, G68A & S79P, that had improved thermal tolerance relative to the exemplary "wild type" enzyme SEQ ID NO:190 (encoded by SEQ ID NO:189), as measured following a heat challenge at 80°C for 20 minutes. Wild-type enzyme and all nine single site amino acid mutants were produced in *E. coli* and purified utilizing an N-terminal hexahistidine tag. There was no noticeable difference in activity due to the tag.

To determine the effect of the single amino acid mutations on enzymatic activity, all nine mutants were purified and their xylanase activity (initial rates at the wild-type temperature optimum, 70°C) was compared to that of the exemplary SEQ ID NO:190 "wild-type" enzyme. Enzyme activities were comparable to wild type (initial rate normalized to 1.0) for D8F, N12L, G17I, G60H, P64V, S65V G68A and S79P mutants (relative initial rates 0.65, 0.68, 0.76, 1.1, 1.0, 1.2, 0.98 and 0.84 respectively) confirming that these mutations do not significantly alter the enzymatic activity. Initial rates were measured 3 or more times and variance was typically less than 10 %. In contrast to these eight mutants, a notable reduction in enzymatic activity was observed for the best thermal tolerant, single site mutant, Q11H (relative initial rate 0.35).

Melting temperature (T_m) of "wild-type" and thermal tolerant single site amino acid mutant enzymes.

The purified SEQ ID NO:190 "wild-type" xylanase and the nine thermal tolerant single site amino acid mutants were analyzed using differential scanning calorimetry (DSC). Aggregation was apparent for the wild-type enzyme as evidenced by a shoulder in the DSC trace for its thermal denaturation, see Figure 6B. The evolved mutant enzymes showed no indication of aggregation. For all enzymes, thermally induced denaturation was irreversible and no discernible transition was observed in a second scan of the sample. Due to the irreversibility of denaturation, only the apparent Tm (melting temperature) could be calculated (as described, e.g., by Sanchez-Ruiz (1992) Biophys. J. 61:921–935; Beldarrain (2000) Biotechnol. Appl. Biochem. 31:77–84). The Tm of the wild-type enzyme was 61°C while the Tm's of all point mutants were increased and ranged from 64°C to 70°C (Figure 6A). The Q11H mutation introduced the largest increase (Tm = 70°C) over wild-type followed by P64V (69°C), G17I (67°C) and D8F (67°C).

15 The "9X" combined GSSM™ exemplary enzyme SEQ ID NO:378

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The "9X" enzyme (SEQ ID NO:378) was constructed by combining the single-site changes of the nine thermal tolerant up-mutants by site-directed mutagenesis (Figure 6A). The "9X" (SEQ ID NO:378) enzyme was expressed in *E. coli* and purified to homogeneity. DSC was performed to determine the melting temperature. The Tm of "9X" enzyme was 34 degrees higher than SEQ ID NO:190, the "wild-type" enzyme, demonstrating a dramatic shift in its thermal stability (Figure 6B).

To evaluate the effect of the combined mutations and elevated melting temperature on the enzyme's biochemical properties, pH and temperature profiles were constructed and compared to SEQ ID NO:190, the "wild-type" enzyme. Figure 7 illustrates the biochemical characterization of "wild type" and "evolved" 9X mutant enzymes. Figure 7A illustrates the pH-dependence of activity for the wild-type and evolved 9X mutant enzymes. Xylanase activity was measured at 37°C at each pH and the initial velocity was plotted against absorbance at 590 nm to determine initial rates. Figure 7B illustrates the temperature-dependence of activity for the wild-type and evolved 9X mutant enzymes. The optimum temperatures of the wild-type and 9X mutant enzymes were measured over a temperature range of 25-100°C at pH 6.0 and are based on initial rates measured over 5 minutes. Figure 7C illustrates the thermal stability of wild-type and evolved 9X mutant enzymes was measured by first heating the enzymes at each of the indicated temperatures for 5

minutes followed by cooling to room temperature and the measurement of residual activity (initial rate at 37°C, pH 6.0). For all experiments initial rates were measured 2 or more times and the variation was less than 10 %.

SEQ ID NO:190 and SEQ ID NO:378 (the "9X" mutant) enzyme had comparable pH/activity profiles with the highest activity between pH 5 and 6 (Figure 7A). Both enzymes had similar initial rate/temperature optima at 70°C, however, SEQ ID NO:190, the "wild-type" enzyme had higher activity at lower temperatures (25-50°C) whereas SEQ ID NO:378 (the "9X" mutant) retained more than 60% of its activity up to 100°C (determined by initial rate) in the presence of substrate (Figure 7B). The activity of SEQ ID NO:190, the "wild-type" enzyme was not detectable at temperatures above 70°C.

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To determine the effect of the 9 combined mutations on enzyme thermal tolerance, residual activity was measured and compared to SEQ ID NO:190, the "wild-type" enzyme. Residual activity was determined by a heat challenge for 5 minutes at each temperature (37, 50, 60, 70, 80 and 90°C) followed by activity measurements at 37°C. SEQ ID NO:190 was completely inactivated above 70°C while the evolved 9X mutant displayed significant activity after heating at 70, 80 and even 90°C (Figure 7C). Furthermore, although the activity of the wild-type enzyme decreased with increasing temperature, the 9X variant was somewhat activated by heating at temperatures up to 60°C.

Generation of combinatorial GSSM™ variants using GeneReassembly™ technology.

To identify combinatorial variants of the 9 single site amino acid mutants with highest thermal tolerance and activity compared to the additively constructed SEQ ID NO:378 (the "9X" variant), a GeneReassemblyTM library (U.S. Patent No. 6,537,776) of all possible mutant combinations (2⁹) was constructed and screened. Using thermal tolerance as the screening criterion, 33 unique combinations of the nine mutations were identified as was the original 9X variant. A secondary screen was performed to select for variants with higher activity/expression than the evolved 9X. This screen yielded 10 variants with sequences possessing between 6 and 8 of the original single mutations in various combinations, as illustrated in Figure 8A. Figure 8 illustrates the combinatorial variants identified using GeneReassemblyTM technology. Figure 8A illustrates the GeneReassemblyTM library of all possible combinations of the 9 GSSMTM point mutations that was constructed and screened for variants with improved thermal tolerance and activity. Eleven variants including the 9X variant were obtained. As shown in the figure, the variants possessed 6, 7, 8, or 9 of the point mutations in various combinations. The corresponding melting temperature transition

midpoint (Tm) determined by DSC of each variant is shown on the right. Figure 8B illustrates the relative activity (initial rate measured over a 5 minute time period) of the 6X-2 and 9X variants compared to wild-type at the temperature optimum (70°C) and pH 6.0. Error bars show the range in the initial rate for 3 measurements.

The melting temperature (T_m) of each of the combinatorial variants was at least 28°C higher than wild type (Figure 8A) and all of the reassembly variants displayed higher relative activity than the 9X enzyme. The activity of one variant in particular, 6X-2, was greater than the wild-type enzyme and significantly better (1.7X) than the 9X enzyme (Figure 8B). Sequence comparison of the reassembly variants identified at least 6 mutations that were required for the enhanced thermostability (>20 degrees). All 33 unique variants found in the initial thermostability screen contained both Q11H and G17I mutations demonstrating their importance for thermal tolerance.

Analysis of wild-type and variant polysaccharide product fingerprints.

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The products generated by the "wild-type," 6X-2 and 9X variants were compared by polysaccharide analysis using carbohydrate gel electrophoresis (PACE). Different substrates (oligosaccharides and polysaccharides) were tested for hydrolysis by the xylanases. The digestion products of the 3 xylanases tested were very similar, as illustrated in Figure 9. All three enzymes hydrolyzed (Xyl)6 and (Xyl)5, mainly into both (Xyl)3 and (Xyl)2, and (Xyl)4 was hydrolyzed to (Xyl)2 (Figure 9A). Only a small amount of hydrolysis of (Xyl)3 into (Xyl)2 and Xyl was observed indicating that (Xyl)3 is a relatively poor substrate for the enzyme. No activity was detected on (Xyl)2. Beechwood xylan, which contains glucuronosyl residues, was hydrolyzed by all three enzymes mainly into (Xyl)2 and (Xyl)3, but other bands were detected that migrated between oligoxylan bands (Figure 9B). In PACE analysis, each oligosaccharide has a specific migration depending on the sugar composition and degree of polymerization (Goubet, F., Jackson, P., Deery, M. and Dupree, P. (2002) *Anal. Biochem. 300*, 53–68), thus, these bands likely correspond to oligoglucuronoxylans. Therefore, the evolved enzymes retained the substrate specificity of the "wild-type" enzyme.

As noted above, Figure 9 illustrates the product fingerprints of "wild-type" SEQ ID NO:190 (encoded by SEQ ID NO:189), 6X-2 (SEQ ID NO:380, encoded by SEQ ID NO:379) and SEQ ID NO:378 (the "9X" mutant) enzyme variant, as determined by PACE. Figure 9A illustrates fingerprints obtained after hydrolysis of oligoxylans (Xyl)3, (Xyl)4, (Xyl)5 and (Xyl)6 by "wild-type" and variant enzymes. Control lanes contain oligosaccharide incubated under the assay conditions in the absence of enzyme. Figure 9B illustrates the

fingerprints obtained after hydrolysis of Beechwood xylan by wild-type and variant enzymes. Standards contained (Xyl)2, (Xyl)3, (Xyl)4. All assays were performed at 37°C and pH 5.5.

A combination of laboratory gene evolution strategies was used to rapidly generate a highly active, thermostable xylanase optimized for process compatibility in a number of industrial market applications. GSSMTM methodology was employed to scan the entire sequence of the exemplary "wild type" xylanase SEQ ID NO:190 (encoded by SEO ID NO:189) and to identify 9 point mutations that improve its thermal tolerance. Although it had no discernable effect on the hydrolysis product profile of the enzyme, as illustrated in Figure 9, the addition of the 9 mutations to the protein sequence resulted in a moderate reduction in enzymatic specific activity at SEQ ID NO:190 (the "wild-type")'s temperature optimum. 70°C, see Figure 9B. Using the GeneReassembly™ method to generate a combinatorial library of the 9 single site amino acid mutants, this reduction in activity was overcome. Ten thermostable variants (Tm's between 89°C and 94°C) with activity better than the "9X" variant were obtained from screening the GeneReassembly I library. With a Tm of 90°C, enzymatic specific activity surpassing wild-type and a product fingerprint unaltered and comparable to SEQ ID NO:190 (the "wild-type"), the 6X-2 variant (SEQ ID NO:380, encoded by SEQ ID NO:379) is particularly notable. To our knowledge the shift in T_m obtained for these variants is the highest increase reported from the application of directed evolution technologies.

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SEQ ID NO:380 (the 6X-2 variant) includes the following changes, as compared to SEQ ID NO:190 (the "wild-type"): D8F, Q11H, G17I, G60H, S65V and G68A. SEQ ID NO:379 includes the following nucleotide changes, as compared to the "wild type" SEQ ID NO:189: the nucleotides at positions 22 to 24 are TTC, the nucleotides at positions 31 to 33 are CAC, the nucleotides at positions 49 to 51 are ATA, the nucleotides at positions 178 to 180 are CAC, the nucleotides at positions 193 to 195 are GTG, the nucleotides at positions 202 to 204 are GCT.

In order to gauge the effectiveness of combinatorial mixing versus addition of the point mutants to the desired phenotype, a fitness parameter combining contributions both from changes in enzyme activity and thermostability was calculated for each mutant. The term fitness as described here is not an objective measure that can be compared to other enzymes, but rather a term that allows the measurement of the success of directed evolution of this particular xylanase. Since enzyme fitness, F, is calculated by equally weighting changes in T_m and enzyme activity for this set of variants, the maximum allowable fitness

value is 2 ($F_{T \le 1}$ and $F_{V \le 1}$, see above). In other words, if the variant with the best activity also had the highest T_m , its fitness value would be 2. With a fitness value near 2 (see Fig. 10B), the 6X-2 variant (SEQ ID NO:380, encoded by SEQ ID NO:379) is the closest to possessing the best possible combination of thermal stability and enzyme activity. The single site mutation that confers the highest value of fitness is S65V. Although the T_m of the S65V mutant is lower than that of the Q11H mutant (66°C verses 70°C respectively), it has a higher fitness value since its specific activity is not reduced relative to wild-type.

Figure 10A is a schematic diagram illustrating the level of thermal stability (represented by Tm) improvement over "wild-type" obtained by GSSMTM evolution. The single site amino acid mutant and the combinatorial variant with the highest thermal stability (Q11H and "9X" (SEQ ID NO:378), respectively) are shown in comparison to wild-type. Figure 10B illustrates a "fitness diagram" of enzyme improvement obtained by combining GSSMTM and GeneReassemblyTM technologies. Fitness was determined using the formula F = FT + FV where fitness (F) is calculated by equally weighting thermal tolerance fitness (FT) and relative activity fitness (FV) as described above. The point mutation that confers the greatest fitness (S65V) is shown. Combining all 9 point mutations also improved fitness (SEQ ID NO:378, the "9X" variant). However, the largest improvement in fitness was obtained by combining GSSMTM and GeneReassemblyTM methods to obtain the best variant, 6X-2 (SEQ ID NO:380).

The GeneReassembly™ method also allowed the identification of important residues that appear absolutely necessary for improved thermal stability. Two key residues, Q11H and G17I, were present in every GeneReassembly™ variant identified based on thermal tolerance (see Figure 6A). The structural determinants for thermal stability of proteins have been studied and several theories have been documented, e.g., by Kinjo (2001) Eur. Biophys. J. 30:378-384; Britton (1999) J. Mol. Biol. 293:1121-1132; Ladenstein (1998) Adv. Biochem. Eng. Biotechnol. 61:37-85; Britton (1995) Eur. J. Biochem. 229:688−695; Tanner (1996) Biochemistry 35:2597−2609; Vetriani (1998) Proc. Natl. Acad. Sci. USA 95:2300−2305. Hydrogen bonding patterns, ionic interactions, hydrophobic packing and decreased length of surface loops are among the key factors even though the contribution of each to protein stability is not fully understood. Given that most of the beneficial point substitutions identified from testing all possible single amino acid substitutions involved the replacement of relatively polar, charged or small (glycine) residues for much larger hydrophobic residues, it can surmised that hydrophobic interactions play the most significant

role in enhancing the thermostability of this protein. Even with a good understanding of the optimal interactions to enhance thermal tolerance, the prediction of where to make mutations that introduce such interactions is not straightforward. A nonrational approach using the GSSMTM method, however, allows rapid sampling of all sidechains at all positions within a protein structure. Such an approach leads to the discovery of amino acid substitutions that introduce functional interactions that could not have been foreseen.

EXAMPLE 6: Pre-treating paper pulp with xylanases of the invention

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In one aspect, xylanases of the invention can be used to pretreat paper pulp. This example describes an exemplary routine screening protocol to determine whether a xylanase is useful in pretreating paper pulp; e.g., in reducing the use of bleaching chemicals (e.g., chlorine dioxide, ClO₂) when used to pretreat Kraft paper pulp.

The screening protocol has two alternative test parameters: Impact of xylanase treatment after an oxygen delignification step (post-O₂ pulp); and, Impact of xylanase in a process that does not include oxygen delignification (pre-O₂ brownstock).

For pulp treatment conditions that simulate process conditions in industrial situations, e.g., factories: pH 8.0; 70 °C; 60 min duration.

The process is schematically depicted in the Flow Diagram of Figure 11.

Twenty xylanases were identified by biochemical tests that were active under these conditions. Of the 20 xylanases, 6 were able to significantly reduce ClO₂ demand when they were used to pretreat Kraft pulp before it was chemically bleached. The six are: SEQ ID NO:182 (encoded by SEQ ID NO:181); SEQ ID NO:160 (encoded by SEQ ID NO:159); SEQ ID NO:198 (encoded by SEQ ID NO:197); SEQ ID NO:168 (encoded by SEQ ID NO:167); SEQ ID NO:216 (encoded by SEQ ID NO:215); SEQ ID NO:260 (encoded by SEQ ID NO:259). Others showed some activity but were not as good. Xylanases SEQ ID NO:182 (encoded by SEQ ID NO:181) and SEQ ID NO:160 (encoded by SEQ ID NO:159) are modular and contain a carbohydrate binding module in addition to the xylanase catalytic domain. It was demonstrated that truncated derivatives of these 2 xylanases containing just the catalytic domain are more effective in this application. The best xylanase, SEQ ID NO:160 (encoded by SEQ ID NO:159) was studied more comprehensively. Results can be summarized as follows:

- pretreatment of post-O₂ spruce/pine/fir (SPF) pulp with 2 units/g of SEQ ID NO:160 (encoded by SEQ ID NO:159) reduces subsequent ClO₂ use by 22% to reach 65%GE brightness;

- pretreatment of pre-O₂ brownstock SPF with 0.5 units/g SEQ ID NO:160 (encoded by SEQ ID NO:159) reduces subsequent ClO₂ use by 13% to reach 65%GE brightness;

- pretreatment of pre-O₂ Aspen pulp with 0.5 units/g SEQ ID NO:160
- 5 (encoded by SEQ ID NO:159) reduces ClO₂ use by at least 22%;
 - pretreatment of pre-O₂ Douglas Fir/Hemlock pulp with 0.5 units/g SEQ ID NO:160 (encoded by SEQ ID NO:159) reduces ClO₂ use by at least 22%;
 - under the treatment conditions employed, the reduction in yield from the xylanase treatment did not exceed 0.5% when compared with pulp that had been bleached at the same kappa factor but not treated with xylanase;
 - optimal conditions for treating post-O₂ SPF pulp with SEQ ID NOS:159, 160 were: pH 6-7, enzyme dose 0.3 units/g, treatment time 20-25 min. Under these conditions, reduction in ClO₂ use of 28% was possible to reach 69%GE brightness.

In further experiments:

- SEQ ID NO:160 (XYLA), encoded by SEQ ID NO: 159 = full length wild type xylanase:
 - XYLA (E.c) = truncated variant of SEQ ID NOS:159, 160 containing only xylanase catalytic domain expressed in *E.coli*
 - XYLA (P.f) = ditto but expressed in P. fluorescens
 SEQ ID NO:182 (encoded by SEQ ID NO: 181) = second full-length wild
 type xylanase:
 - XYLB (E.c) = truncated variant etc, etc expressed in *E.coli*
 - XYLB (P.f) = ditto but expressed in P. fluorescens

Dose Response Data for Lead Xylanases on Pre-O2 Brownstock

25 <u>Conditions for xylanase stage (X-stage) as follows:</u>

9H 8

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Temperature 70°C

Time 60 min

Kappa factor 0.24

For no-enzyme control, kappa factor was 0.30

Results showed a dose dependent increase in brightness for xylanase-treated samples at a lower charge of chlorine dioxide (ClO₂) (Kf 0.24 vs Kf 0.30).

In each case, the truncated derivative looked to be more effective that the full-length xylanase. Optimal xylanase dose looked to be around 0.6 to 0.7 U/g pulp.

Pretreatment of Intercontinental Pre-O2 Brownstock with the best 4 Xylanases

Determination of ClO₂ Dose Response in D₀

Experimental outline

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- Pre-O₂ Brownstock
 - o Initial kappa 31.5
- X stage conditions
 - o Xylanase charge 0.7 U/gm
 - o Temperature 70°C
 - o pH 8
 - o Treatment time 1 hr
 - o Pulp consistency 10%
- Bleach sequence XDE_p
 - o Kappa factor 0.22, 0.26 and 0.30 (%D on pulp: 2.63, 3.12 and 3.60)

Final brightness after 3-stage bleach sequence versus Kappa factor (ClO₂ charge):

- XYLB At 61.5 final brightness, X-stage enables reduction in ClO₂ use of 3.89 kg/ton pulp.
- XYLB (E.c) At 61.5 final brightness, X-stage enables reduction in ClO₂ charge of 4.07 kg/ton pulp.
 - XYLA At 61.5 brightness, X-stage enables a reduction in ClO₂ use of 4.07 kg/ton pulp.
 - XYLA (E.c) At 61.5 final brightness, X-stage enables reduction in ClO₂ use of 4.90 kg/ton pulp.
- 25 Determination of ClO₂ Dose Response in D₀:

Enzyme	CIO₂ Savings in D₀ (kg/ton OD)	Kf reduction in D₀
XYLB	3.89	11.7%
XYLB (E.c)	5.08	15.8%
XYLA	4.07	12.2%
XYLA (E.c)	4.90	14.7%

Xylanase 0.7 U/g, pH 8.0, 70 °C, 1 hr

Pulp: Pre-O₂ Brownstock, initial kappa 31.5

Percentage saving of ClO₂ is of little significance to the industry. Their

primary concern is lbs of ClO₂ required per ton OD pulp. This makes sense when one considers that a lower percentage saving seen with a high initial kappa brownstock can be more valuable in terms of lbs of ClO₂ saved than a higher percentage reduction for a low initial kappa pulp which will require a lower total charge of ClO₂ to reach target brightness.

Relationship between Brightness, Yield and Kappa Factor for Bleached Control Pulp:

The results showed that bleaching with increasing doses of ClO₂ to achieve higher target brightness results in increased loss of pulp yield. This is an issue because pulp at this stage of the process has a value of almost \$400 per ton and loss of cellulose costs money.

A benefit of xylanase (e.g., a xylanase of the invention) is that use of a lower ClO₂ dose can reduce yield losses as long as the action of the xylanase itself doesn't cancel out the gain.

<u>Dose Response Data for Pretreatment of Pre-O₂ Brownstock with Xylanase XYLB (P.f):</u> Experimental outline

- Northwood Pre-O2 Brownstock
- -Initial kappa 28.0

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- -Initial consistency 32.46%
 - -Initial brightness 28.37
 - X stage conditions
 - -Xylanase charge 0 to 2.70 U/gm
 - -Temperature 58°C to 61°C

- -pH 8.2 to 8.5
- -Treatment time 1hr
- •Bleach sequence XDEp
- -Kappa factor 0.24
- •ClO₂ saving calculated for Kappa factors between 0.24 and 0.30

The purpose of this experiment was to evaluate the best of the 4 xylanases on unwashed SPF brownstock. Results showed dose-dependent increases in final brightness for pulp treated with XYLB (E.c), with brightness achieved in presence of xylanase at lower Kf of 0.24, approaching brightness achieved at higher Kf of 0.30 asymptotically.

10 Relationship between Dose of Xylanase XYLB (E.c) and Chlorine Dioxide Saving (Pre-O2 Brownstock):

CIO ₂ Saving in % OD Pulp	CIO ₂ Saving in kg/ton Pulp	Xylanase Dose in U/gm
0.299%	2.99	0.31
0.363%	3.63	0.51
0.406%	4.06	0.71
0.439%	4.39	0.91
0.483%	4.83	1.26
0.523%	5.32	1.80
0.587%	5.87	2.70

Optimum Xylanase Dose is between 0.5 and 0.9 U/gm

The optimum dose lies in the range 0.5 to 0.9 U/g. Above this dose there is a diminishing return per unit increment of xylanase. Reductions in chlorine dioxide dose per ton of pulp treated of this magnitude are commercially significant.

Three-stage biobleaching procedure

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A three-stage biobleaching procedure was developed that would closely simulate the actual bleaching operations in a pulp mill bleach plant (Fig. 1). This bleach sequence is designated by (X)DoEp, in which \underline{X} represents the xylanase treatment stage, \underline{D} for chlorine dioxide bleaching stage, and \underline{Ep} for alkaline peroxide extraction stage. The primary feedstock used in our application tests was Southern Softwood Kraft Brownstock (without oxygen delignification). The most effective xylanase candidates that showed high

bleach chemical reduction potential in the biobleaching assays were also tested on two species of hardwood Kraft pulp (maple and aspen). Upon completion of each biobleaching round, the ensuing pulp was used to produce TAPPI (Technical Association of Pulp and Paper Industries)-standard handsheets. The GE% brightness of each handsheet was measured, and the brightness values were used as the indication of how well each enzyme had performed on the pulp during the enzymatic pretreatment stage (X). Results:

Out of approximately 110 xylanases that were screened using the (X)DoEp biobleaching sequence, 4 enzymes, i.e., XYLA (P.f); XYLB (P.f); SEQ ID NO216 (encoded by SEQ ID NO:215); SEQ ID NO:176 (encoded by SEQ ID NO: 175); showed the greatest potential for reducing the use of bleaching chemicals. While XYLA (P.f) and XYLB (P.f) exhibited equally high performance (best among the four good performers), XYLA (P.f) showed a better pH tolerance than XYLB (P.f). The results can be summarized as follows:

- It is possible to achieve a handsheet brightness of 60 (GE%) using a three-stage bleach sequence [(X)DoEp] that involves pretreatment of Southern Softwood Kraft Brownstock with the following four enzymes at the loading levels listed below (pH=8, 65 °C & 1 h):
 - o XYLA (P.f) at 0.55 U/g pulp

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- o XYLB (P.f) at 0.75 U/g pulp
- o SEQ ID NOS:215, 216 at 1.80 U/g pulp
- SEQ ID NOS:175, 176 at 1.98 U/g pulp
- Pretreatment of Southern Softwood Kraft Brownstock with 2 U/g pulp of XYLA (P.f) reduces ClO₂ use by 18.7% to reach a final GE% brightness of 61.
- XYLA (P.f) exhibits good tolerance at higher pH and provides more than 14% chemical savings when the enzymatic pretreatment stage is run at pH=10.
- Pretreatment of Southern Softwood Kraft Brownstock with 2 U/g pulp of XYLB (P.f) reduces ClO₂ use by 16.3% to reach a final GE% brightness of 60.5.
 - Pretreatment of aspen Kraft pulp with 2 U/g pulp of XYLA (P.f) and XYLB (P.f) reduces ClO₂ use by about 35% to reach a final GE% brightness of 77.
- Pretreatment of maple Kraft pulp with 2 U/g pulp of XYLA (P.f) and XYLB (P.f)
 reduces ClO₂ use by about 38% to reach a final GE% brightness of 79.

 The two best performing xylanases, namely XYLA (P.f) and XYLB (P.f), are truncated enzymes, containing just the catalytic domain, and were produced in *Pseudomonas* fluorescens.

While the invention has been described in detail with reference to certain preferred aspects thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.

WHAT IS CLAIMED IS:

An isolated or recombinant nucleic acid comprising a nucleic acid 1. sequence having at least 50% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID 5 NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEO ID NO:29, SEO ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEO ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID 10 NO:61, SEO ID NO:63, SEO ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEO ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID 15 NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEO ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEO ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID 20 NO:165, SEO ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID 25 NO:215, SEO ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEO ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEO ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEO ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID 30 NO:265, SEO ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEO ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEO ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID

NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379, over a region of at least about 100 residues, wherein the nucleic acid encodes at least one polypeptide having a xylanase activity, and the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection.

2. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63% or 64%.

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3. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is at least about 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID 20 NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEO ID NO:25, SEO ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEO ID NO:47, SEO ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID 25 NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEO ID NO:69, SEO ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEO ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID 30 NO:111, SEO ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID

NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEO ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEO ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEO ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379.

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- 4. The isolated or recombinant nucleic acid of claim 1, wherein the sequence identity is over a region of at least about 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150 or more residues, or the full length of a gene or a transcript.
- 5. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence comprises a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEO ID NO:27, SEO ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID

NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:13 NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID 10 NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID 15 NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:208, SEQ ID NO:20 NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID 20 NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID 25 NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:321, SEQ ID NO:321, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:32 NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:331, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:33 NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID 30 NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379.

6. The isolated or recombinant nucleic acid of claim 1, wherein the nucleic acid sequence encodes a polypeptide having a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEO ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEO ID NO:26, SEO ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEO ID NO:38, SEO ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEO ID NO:48, SEO ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEO ID NO:70, SEO ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID 10 NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEO ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID 15 NO:132; SEQ ID NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; SEQ ID NO:142; SEO ID NO:144; NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEO ID NO:154, SEO ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEO ID NO:164, SEO ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEO ID NO:174, SEO ID NO:176, SEO ID NO:178, SEO ID NO:180, SEO ID NO:182, 20 SEO ID NO:184, SEO ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, 25 SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, SEQ ID NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278, SEQ ID NO:280, SEQ ID NO:282, 30 SEQ ID NO:284, SEQ ID NO:286, SEQ ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, SEQ ID NO:320, SEQ ID NO:322,

SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID NO:334, SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376, SEQ ID NO:378 or SEQ ID NO:380.

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- 7. The isolated or recombinant nucleic acid of claim 1, wherein the sequence comparison algorithm is a BLAST version 2.2.2 algorithm where a filtering setting is set to blastall -p blastp -d "nr pataa" -F F, and all other options are set to default.
- 8. The isolated or recombinant nucleic acid of claim 1, wherein the xylanase activity comprises catalyzing hydrolysis of internal β -1,4-xylosidic linkages.
- 15 9. The isolated or recombinant nucleic acid of claim 8, wherein the xylanase activity comprises an endo-1,4-beta-xylanase activity.
 - 10. The isolated or recombinant nucleic acid of claim 1, wherein the xylanase activity comprises hydrolyzing a xylan to produce a smaller molecular weight xylose and xylo-oligomer.
 - 11. The isolated or recombinant nucleic acid of claim 10, wherein the xylan comprises an arabinoxylan.
- 25 12. The isolated or recombinant nucleic acid of claim 11, wherein the arabinoxylan comprises a water soluble arabinoxylan.
 - 13. The isolated or recombinant nucleic acid of claim 12, wherein the water soluble arabinoxylan comprises a dough or a bread product.
 - 14. The isolated or recombinant nucleic acid of claim 1, wherein the xylanase activity comprises hydrolyzing polysaccharides comprising 1,4-β-glycoside-linked D-xylopyranoses.

15. The isolated or recombinant nucleic acid of claim 1, wherein the xylanase activity comprises hydrolyzing hemicelluloses.

- The isolated or recombinant nucleic acid of claim 15, wherein the
 xylanase activity comprises hydrolyzing hemicelluloses in a wood or paper pulp or a paper product.
 - 17. The isolated or recombinant nucleic acid of claim 8, wherein the xylanase activity comprises catalyzing hydrolysis of xylans in a feed or a food product.

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- 18. The isolated or recombinant nucleic acid of claim 17, wherein the feed or food product comprises a cereal-based animal feed, a wort or a beer, a milk or a milk product, a fruit or a vegetable.
- 15. The isolated or recombinant nucleic acid of claim 1, wherein the xylanase activity comprises catalyzing hydrolysis of xylans in a microbial cell or a plant cell.
 - 20. The isolated or recombinant nucleic acid of claim 1, wherein the xylanase activity is thermostable.
 - 21. The isolated or recombinant nucleic acid of claim 20, wherein the polypeptide retains a xylanase activity under conditions comprising a temperature range of between about 37°C to about 95°C, or between about 55°C to about 85°C, or between about 70°C to about 95°C, or between about 90°C to about 95°C.
 - 22. The isolated or recombinant nucleic acid of claim 1, wherein the xylanase activity is thermotolerant.
- The isolated or recombinant nucleic acid of claim 22, wherein the polypeptide retains a xylanase activity after exposure to a temperature in the range from greater than 37°C to about 95°C, from greater than 55°C to about 85°C, or between about 70°C to about 75°C, or from greater than 90°C to about 95°C.

An isolated or recombinant nucleic acid, wherein the nucleic acid 24. comprises a sequence that hybridizes under stringent conditions to a nucleic acid comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, 5 SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEO ID NO:57, SEO ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEO ID NO:79, SEO ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID 10 NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEO ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEO ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, 15 SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, 20 SEO ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEO ID NO:221, SEO ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, 25 SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEO ID NO:261, SEO ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEO ID NO:281, SEO ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, 30 SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEO ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329,

SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379, wherein the nucleic acid encodes a polypeptide having a xylanase activity.

- 25. The isolated or recombinant nucleic acid of claim 24, wherein the nucleic acid is at least about 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more residues in length or the full length of the gene or transcript.
- 26. The isolated or recombinant nucleic acid of claim 24, wherein the stringent conditions include a wash step comprising a wash in 0.2X SSC at a temperature of about 65°C for about 15 minutes.

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A nucleic acid probe for identifying a nucleic acid encoding a 27. polypeptide with a xylanase activity, wherein the probe comprises at least 10 consecutive bases of a sequence comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, 20 SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID ${\tt NO:63, SEQ\:ID\:NO:65, SEQ\:ID\:NO:67, SEQ\:ID\:NO:69, SEQ\:ID\:NO:71, SEQ\:ID\:NO:73,}$ SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID 25 NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID ID NO:107, SEO ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID 30 NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID

NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:18 NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:27 10 NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:28 NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:309, SEQ ID NO:300, SEQ ID NO:30 NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID 15 NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:34 NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID 20 NO:377 or SEQ ID NO:379, wherein the probe identifies the nucleic acid by binding or hybridization.

- 28. The nucleic acid probe of claim 27, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.
- 29. A nucleic acid probe for identifying a nucleic acid encoding a polypeptide having a xylanase activity, wherein the probe comprises a nucleic acid comprising at least about 10 consecutive residues of a nucleic acid sequence having at least 50% sequence identity to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41,

SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, S NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, S NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, S NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:14 10 NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:16 NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:18 NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID 15 NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:20 NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:21 NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID 20 NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID 25 NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:31 NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:32 NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:355, SEQ ID NO:35 NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID 30 NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:36 NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID

NO:377 or SEQ ID NO:379, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by visual inspection.

- 30. The nucleic acid probe of claim 29, wherein the probe comprises an oligonucleotide comprising at least about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, about 60 to 100, or about 50 to 150 consecutive bases.
 - 31. An amplification primer pair for amplifying a nucleic acid encoding a polypeptide having a xylanase activity, wherein the primer pair is capable of amplifying a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.

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- 32. The amplification primer pair of claim 31, wherein a member of the amplification primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 consecutive bases of the sequence, or, about 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more consecutive bases of the sequence.
- 33. An amplification primer pair, wherein the primer pair comprises a first member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of SEQ ID NO:1, SEQ ID 20 NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID 25 NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID 30 NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID

NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEO ID NO:195, SEO ID NO:197, SEO ID NO:199, SEO ID NO:201, SEO ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID 10 NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID 15 NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID 20 NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379, and a second member having a sequence as set forth by about the first (the 5') 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 or more residues of the complementary strand of the first 25 member.

- 34. A xylanase-encoding nucleic acid generated by amplification of a polynucleotide using an amplification primer pair as set forth in claim 33.
- 35. The xylanase-encoding nucleic acid of claim 34, wherein the amplification is by polymerase chain reaction (PCR).

36. The xylanase-encoding nucleic acid of claim 34, wherein the nucleic acid generated by amplification of a gene library.

- 37. The xylanase-encoding nucleic acid of claim 34, wherein the gene library is an environmental library.
 - 38. An isolated or recombinant xylanase encoded by a xylanase-encoding nucleic acid as set forth in claim 34.
- 39. A method of amplifying a nucleic acid encoding a polypeptide having a xylanase activity comprising amplification of a template nucleic acid with an amplification primer sequence pair capable of amplifying a nucleic acid sequence as set forth in claim 1 or claim 24, or a subsequence thereof.
- 15 40. An expression cassette comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.
 - 41. A vector comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

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42. A cloning vehicle comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24, wherein the cloning vehicle comprises a viral vector, a plasmid, a phage, a phagemid, a cosmid, a fosmid, a bacteriophage or an artificial chromosome.

- 43. The cloning vehicle of claim 42, wherein the viral vector comprises an adenovirus vector, a retroviral vector or an adeno-associated viral vector.
- 44. The cloning vehicle of claim 42, comprising a bacterial artificial chromosome (BAC), a plasmid, a bacteriophage P1-derived vector (PAC), a yeast artificial chromosome (YAC), or a mammalian artificial chromosome (MAC).
 - 45. A transformed cell comprising a nucleic acid comprising a sequence as set forth in claim 1 or claim 24.

46. A transformed cell comprising an expression cassette as set forth in claim 40.

- 5 47. The transformed cell of claim 40, wherein the cell is a bacterial cell, a mammalian cell, a fungal cell, a yeast cell, an insect cell or a plant cell.
 - 48. A transgenic non-human animal comprising a sequence as set forth in claim 1 or claim 24.
 - 49. The transgenic non-human animal of claim 48, wherein the animal is a mouse.
- 50. A transgenic plant comprising a sequence as set forth in claim 1 or claim 24.

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- 51. The transgenic plant of claim 50, wherein the plant is a corn plant, a sorghum plant, a potato plant, a tomato plant, a wheat plant, an oilseed plant, a rapeseed plant, a soybean plant, a rice plant, a barley plant, a grass, or a tobacco plant.
- 52. A transgenic seed comprising a sequence as set forth in claim 1 or claim 24.
- 53. The transgenic seed of claim 52, wherein the seed is a corn seed, a wheat kernel, an oilseed, a rapeseed, a soybean seed, a palm kernel, a sunflower seed, a sesame seed, a rice, a barley, a peanut or a tobacco plant seed.
- 54. An antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24, or a subsequence thereof.
 - 55. The antisense oligonucleotide of claim 49, wherein the antisense oligonucleotide is between about 10 to 50, about 20 to 60, about 30 to 70, about 40 to 80, or about 60 to 100 bases in length.

56. A method of inhibiting the translation of a xylanase message in a cell comprising administering to the cell or expressing in the cell an antisense oligonucleotide comprising a nucleic acid sequence complementary to or capable of hybridizing under stringent conditions to a sequence as set forth in claim 1 or claim 24.

57. A double-stranded inhibitory RNA (RNAi) molecule comprising a subsequence of a sequence as set forth in claim 1 or claim 24.

- 10 58. The double-stranded inhibitory RNA (RNAi) molecule of claim 52, wherein the RNAi is about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more duplex nucleotides in length.
- 59. A method of inhibiting the expression of a xylanase in a cell comprising administering to the cell or expressing in the cell a double-stranded inhibitory RNA (iRNA), wherein the RNA comprises a subsequence of a sequence as set forth in claim 1 or claim 24.
- 60. An isolated or recombinant polypeptide (i) having at least 50% sequence identity to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID 20 NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID 25 NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID 30 NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132; SEQ ID NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; SEQ ID NO:142; SEQ ID NO:144; NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158,

SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, 10 SEQ ID NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278, SEQ ID NO:280, SEQ ID NO:282, SEQ ID NO:284, SEQ ID NO:286, SEQ ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296, SEQ ID NO:298, 15 SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, SEQ ID NO:320, SEQ ID NO:322, SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID NO:334, SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, 20 SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376, SEQ ID NO:378 or SEQ ID NO:380, over a region of at least about 100 residues, wherein the sequence identities are determined by analysis with a sequence comparison algorithm or by a visual inspection, 25 or, (ii) encoded by a nucleic acid having at least 50% sequence identity to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, 30 SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID

NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID 5 NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID 10 NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID 15 NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID 20 NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID 25 NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379, over a region of at least about 100 residues, and the sequence identities are 30 determined by analysis with a sequence comparison algorithm or by a visual inspection, or encoded by a nucleic acid capable of hybridizing under stringent conditions to a sequence as set forth in SEO ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEO ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID

NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID 10 NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID 15 NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID 20 NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID 25 NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID 30 NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID

NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379.

61. The isolated or recombinant polypeptide of claim 60, wherein the sequence identity is over a region of at least about at least about 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, or is 100% sequence identity.

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62. The isolated or recombinant polypeptide of claim 60, wherein the sequence identity is over a region of at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050 or more residues, or the full length of an enzyme.

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63. The isolated or recombinant polypeptide of claim 60, wherein the polypeptide has a sequence as set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEO ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62. SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132; SEQ ID NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; SEQ ID NO:142; SEQ ID NO:144; NO:146. SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156. SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, SEQ ID NO:186,

SEQ ID NO:188, SEQ ID NO:190, SEQ ID NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID NO:242, SEQ ID NO:244, SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, SEQ ID NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278, SEQ ID NO:280, SEQ ID NO:282, SEQ ID NO:284, SEQ ID NO:286, SEQ ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296. SEQ ID NO:298, SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, SEQ ID NO:320, SEQ ID NO:322, SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID NO:334, SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376. SEQ ID NO:378 or SEQ ID NO:380.

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- 64. The isolated or recombinant polypeptide of claim 60, wherein the polypeptide has a xylanase activity.
- 25 65. The isolated or recombinant polypeptide of claim 64, wherein the xylanase activity comprises catalyzing hydrolysis of internal β-1,4-xylosidic linkages.
 - 66. The isolated or recombinant polypeptide of claim 65, wherein the xylanase activity comprises an endo-1,4-beta-xylanase activity.
 - 67. The isolated or recombinant polypeptide of claim 64, wherein the xylanase activity comprises hydrolyzing a xylan to produce a smaller molecular weight xylose and xylo-oligomer.

68. The isolated or recombinant polypeptide of claim 67, wherein the xylan comprises an arabinoxylan.

- 69. The isolated or recombinant polypeptide of claim 68, wherein the arabinoxylan comprises a water soluble arabinoxylan.
 - 70. The isolated or recombinant polypeptide of claim 69, wherein the water soluble arabinoxylan comprises a dough or a bread product.
- 71. The isolated or recombinant polypeptide of claim 64, wherein the xylanase activity comprises hydrolyzing polysaccharides comprising 1,4-β-glycoside-linked D-xylopyranoses.
- 72. The isolated or recombinant polypeptide of claim 64, wherein the xylanase activity comprises hydrolyzing hemicelluloses.
 - 73. The isolated or recombinant polypeptide of claim 72, wherein the xylanase activity comprises hydrolyzing hemicelluloses in a wood or paper pulp or a paper product.

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- 74. The isolated or recombinant polypeptide of claim 73, wherein the xylanase activity comprises catalyzing hydrolysis of xylans in a feed or a food product.
- 75. The isolated or recombinant polypeptide of claim 74, wherein the feed or food product comprises a cereal-based animal feed, a wort or a beer, a milk or a milk product, a fruit or a vegetable.
 - 76. The isolated or recombinant polypeptide of claim 64, wherein the xylanase activity comprises catalyzing hydrolysis of xylans in a microbial cell or a plant cell.
 - 77. The isolated or recombinant polypeptide of claim 64, wherein the xylanase activity is thermostable.

78. The isolated or recombinant polypeptide of claim 77, wherein the polypeptide retains a xylanase activity under conditions comprising a temperature range of between about 1°C to about 5°C, between about 5°C to about 15°C, between about 15°C to about 25°C, between about 25°C to about 37°C, between about 37°C to about 95°C, between about 55°C to about 85°C, between about 70°C to about 95°C, between about 70°C to about 75°C, or between about 90°C to about 95°C.

79. The isolated or recombinant polypeptide of claim 64, wherein the xylanase activity is thermotolerant.

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- 80. The isolated or recombinant polypeptide of claim 79, wherein the polypeptide retains a xylanase activity after exposure to a temperature in the range from between about 1°C to about 5°C, between about 15°C to about 15°C, between about 15°C to about 25°C, between about 25°C to about 37°C, between about 37°C to about 95°C, between about 95°C, or between about 90°C to about 95°C, or more.
- 81. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 60 and lacking a signal sequence or a prepro sequence.
- 82. An isolated or recombinant polypeptide comprising a polypeptide as set forth in claim 60 and having a heterologous signal sequence or a heterologous prepro sequence.
- 25 83. The isolated or recombinant polypeptide of claim 64, wherein the xylanase activity comprises a specific activity at about 37°C in the range from about 100 to about 1000 units per milligram of protein, from about 500 to about 750 units per milligram of protein, from about 500 to about 1200 units per milligram of protein, or from about 750 to about 1000 units per milligram of protein.
 - 84. The isolated or recombinant polypeptide of claim 79, wherein the thermotolerance comprises retention of at least half of the specific activity of the xylanase at 37°C after being heated to an elevated temperature.

85. The isolated or recombinant polypeptide of claim 79, wherein the thermotolerance comprises retention of specific activity at 37°C in the range from about 500 to about 1200 units per milligram of protein after being heated to an elevated temperature.

- 5 86. The isolated or recombinant polypeptide of claim 60, wherein the polypeptide comprises at least one glycosylation site.
 - 87. The isolated or recombinant polypeptide of claim 86, wherein the glycosylation is an N-linked glycosylation.

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- 88. The isolated or recombinant polypeptide of claim 87, wherein the polypeptide is glycosylated after being expressed in a *P. pastoris* or a *S. pombe*.
- 89. The isolated or recombinant polypeptide of claim 64, wherein the polypeptide retains a xylanase activity under conditions comprising about pH 6.5, pH 6.0, pH 5.5, 5.0, pH 4.5 or 4.0.
- 90. The isolated or recombinant polypeptide of claim 64, wherein the polypeptide retains a xylanase activity under conditions comprising about pH 7.5, pH 8.0, pH 20 8.5, pH 9, pH 9.5, pH 10 or pH 10.5.
 - 91. A protein preparation comprising a polypeptide as set forth in claim 60, wherein the protein preparation comprises a liquid, a solid or a gel.
- 25 92. A heterodimer comprising a polypeptide as set forth in claim 60 and a second domain.
 - 93. The heterodimer of claim 92, wherein the second domain is a polypeptide and the heterodimer is a fusion protein.
 - 94. The heterodimer of claim 92, wherein the second domain is an epitope or a tag.
 - 95. A homodimer comprising a polypeptide as set forth in claim 60.

96. An immobilized polypeptide, wherein the polypeptide comprises a sequence as set forth in claim 60, or a subsequence thereof.

- 5 97. The immobilized polypeptide of claim 96, wherein the polypeptide is immobilized on a cell, a metal, a resin, a polymer, a ceramic, a glass, a microelectrode, a graphitic particle, a bead, a gel, a plate, an array or a capillary tube.
- 98. An array comprising an immobilized polypeptide as set forth in claim 10 60.
 - 99. An array comprising an immobilized nucleic acid as set forth in claim 1 or claim 24.
- 15 100. An isolated or recombinant antibody that specifically binds to a polypeptide as set forth in claim 60.

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- 101. The isolated or recombinant antibody of claim 100, wherein the antibody is a monoclonal or a polyclonal antibody.
- 102. A hybridoma comprising an antibody that specifically binds to a polypeptide as set forth in claim 60.
- 103. A method of isolating or identifying a polypeptide with a xylanase activity comprising the steps of:
 - (a) providing an antibody as set forth in claim 100;
 - (b) providing a sample comprising polypeptides; and
 - (c) contacting the sample of step (b) with the antibody of step (a) under conditions wherein the antibody can specifically bind to the polypeptide, thereby isolating or identifying a polypeptide having a xylanase activity.
 - 104. A method of making an anti-xylanase antibody comprising administering to a non-human animal a nucleic acid as set forth in claim 1 or claim 24 or a

subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-xylanase antibody.

- 105. A method of making an anti-xylanase antibody comprising

 5 administering to a non-human animal a polypeptide as set forth in claim 60 or a subsequence thereof in an amount sufficient to generate a humoral immune response, thereby making an anti-xylanase antibody.
- 106. A method of producing a recombinant polypeptide comprising the steps of: (a) providing a nucleic acid operably linked to a promoter, wherein the nucleic acid comprises a sequence as set forth in claim 1 or claim 24; and (b) expressing the nucleic acid of step (a) under conditions that allow expression of the polypeptide, thereby producing a recombinant polypeptide.
 - 107. The method of claim 106, further comprising transforming a host cell with the nucleic acid of step (a) followed by expressing the nucleic acid of step (a), thereby producing a recombinant polypeptide in a transformed cell.
- 108. A method for identifying a polypeptide having a xylanase activity comprising the following steps:
 - (a) providing a polypeptide as set forth in claim 64;
 - (b) providing a xylanase substrate; and

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- (c) contacting the polypeptide with the substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of a reaction product, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product detects a polypeptide having a xylanase activity.
- 109. A method for identifying a xylanase substrate comprising the following steps:
 - (a) providing a polypeptide as set forth in claim 64;
 - (b) providing a test substrate; and
- (c) contacting the polypeptide of step (a) with the test substrate of step (b) and detecting a decrease in the amount of substrate or an increase in the amount of reaction

product, wherein a decrease in the amount of the substrate or an increase in the amount of a reaction product identifies the test substrate as a xylanase substrate.

- 110. A method of determining whether a test compound specifically binds
 to a polypeptide comprising the following steps:
 - (a) expressing a nucleic acid or a vector comprising the nucleic acid under conditions permissive for translation of the nucleic acid to a polypeptide, wherein the nucleic acid has a sequence as set forth in claim 1 or claim 24;
 - (b) providing a test compound;

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- (c) contacting the polypeptide with the test compound; and
- (d) determining whether the test compound of step (b) specifically binds to the polypeptide.
- 111. A method of determining whether a test compound specifically binds 15 to a polypeptide comprising the following steps:
 - (a) providing a polypeptide as set forth in claim 60;
 - (b) providing a test compound;
 - (c) contacting the polypeptide with the test compound; and
- (d) determining whether the test compound of step (b) specifically binds to the 20 polypeptide.
 - 112. A method for identifying a modulator of a xylanase activity comprising the following steps:
 - (a) providing a polypeptide as set forth in claim 64;
 - (b) providing a test compound;
 - (c) contacting the polypeptide of step (a) with the test compound of step (b) and measuring an activity of the xylanase, wherein a change in the xylanase activity measured in the presence of the test compound compared to the activity in the absence of the test compound provides a determination that the test compound modulates the xylanase activity.
 - 113. The method of claim 112, wherein the xylanase activity is measured by providing a xylanase substrate and detecting a decrease in the amount of the substrate or an

increase in the amount of a reaction product, or, an increase in the amount of the substrate or a decrease in the amount of a reaction product.

- 114. The method of claim 113, wherein a decrease in the amount of the substrate or an increase in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an activator of a xylanase activity.
- 115. The method of claim 113, wherein an increase in the amount of the substrate or a decrease in the amount of the reaction product with the test compound as compared to the amount of substrate or reaction product without the test compound identifies the test compound as an inhibitor of a xylanase activity.
- 116. A computer system comprising a processor and a data storage device
 wherein said data storage device has stored thereon a polypeptide sequence or a nucleic acid
 sequence, wherein the polypeptide sequence comprises sequence as set forth in claim 60, a
 polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.
- The computer system of claim 115, further comprising a sequence
 comparison algorithm and a data storage device having at least one reference sequence stored thereon.
 - 118. The computer system of claim 117, wherein the sequence comparison algorithm comprises a computer program that indicates polymorphisms.
 - 119. The computer system of claim 117, further comprising an identifier that identifies one or more features in said sequence.

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120. A computer readable medium having stored thereon a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 60; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24.

121. A method for identifying a feature in a sequence comprising the steps of: (a) reading the sequence using a computer program which identifies one or more features in a sequence, wherein the sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 60; a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) identifying one or more features in the sequence with the computer program.

A method for comparing a first sequence to a second sequence 122. comprising the steps of: (a) reading the first sequence and the second sequence through use of a computer program which compares sequences, wherein the first sequence comprises a polypeptide sequence or a nucleic acid sequence, wherein the polypeptide sequence comprises a polypeptide as set forth in claim 60 or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and (b) determining differences between the first sequence and the second sequence with the computer program.

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123. The method of claim 122, wherein the step of determining differences between the first sequence and the second sequence further comprises the step of identifying polymorphisms.

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The method of claim 123, further comprising an identifier that 124. identifies one or more features in a sequence.

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a computer program and identifying one or more features in the sequence.

The method of claim 124, comprising reading the first sequence using

- 126. A method for isolating or recovering a nucleic acid encoding a polypeptide with a xylanase activity from an environmental sample comprising the steps of:
- (a) providing an amplification primer sequence pair as set forth in claim 31 or claim 33:

- (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to the amplification primer pair; and,
- (c) combining the nucleic acid of step (b) with the amplification primer pair of step (a) and amplifying nucleic acid from the environmental sample, thereby isolating or

recovering a nucleic acid encoding a polypeptide with a xylanase activity from an environmental sample.

The method of claim 126, wherein each member of the amplification 127. primer sequence pair comprises an oligonucleotide comprising at least about 10 to 50 5 consecutive bases of a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID 10 NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, 15 SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID 20 NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID 25 NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID 30 NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID

NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379, or a subsequence thereof.

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- 128. A method for isolating or recovering a nucleic acid encoding a polypeptide with a xylanase activity from an environmental sample comprising the steps of:
- (a) providing a polynucleotide probe comprising a sequence as set forth in claim 1 or claim 24, or a subsequence thereof;
- (b) isolating a nucleic acid from the environmental sample or treating the environmental sample such that nucleic acid in the sample is accessible for hybridization to a polynucleotide probe of step (a);
- (c) combining the isolated nucleic acid or the treated environmental sample of step (b) with the polynucleotide probe of step (a); and
- (d) isolating a nucleic acid that specifically hybridizes with the polynucleotide probe of step (a), thereby isolating or recovering a nucleic acid encoding a polypeptide with a xylanase activity from an environmental sample.
- 129. The method of claim 127 or claim 128, wherein the environmental sample comprises a water sample, a liquid sample, a soil sample, an air sample or a biological sample.
 - 130. The method of claim 129, wherein the biological sample is derived from a bacterial cell, a protozoan cell, an insect cell, a yeast cell, a plant cell, a fungal cell or a mammalian cell.
 - 131. A method of generating a variant of a nucleic acid encoding a polypeptide with a xylanase activity comprising the steps of:

(a) providing a template nucleic acid comprising a sequence as set forth in claim 1 or claim 24; and

(b) modifying, deleting or adding one or more nucleotides in the template sequence, or a combination thereof, to generate a variant of the template nucleic acid.

132. The method of claim 131, further comprising expressing the variant nucleic acid to generate a variant xylanase polypeptide.

133. The method of claim 131, wherein the modifications, additions or deletions are introduced by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSMTM), synthetic ligation reassembly (SLR) and a combination thereof.

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- deletions are introduced by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.
- 25 135. The method of claim 131, wherein the method is iteratively repeated until a xylanase having an altered or different activity or an altered or different stability from that of a polypeptide encoded by the template nucleic acid is produced.
- 136. The method of claim 135, wherein the variant xylanase polypeptide is 30 thermotolerant, and retains some activity after being exposed to an elevated temperature.
 - 137. The method of claim 135, wherein the variant xylanase polypeptide has increased glycosylation as compared to the xylanase encoded by a template nucleic acid.

138. The method of claim 135, wherein the variant xylanase polypeptide has a xylanase activity under a high temperature, wherein the xylanase encoded by the template nucleic acid is not active under the high temperature.

- 5 139. The method of claim 131, wherein the method is iteratively repeated until a xylanase coding sequence having an altered codon usage from that of the template nucleic acid is produced.
- 140. The method of claim 131, wherein the method is iteratively repeated until a xylanase gene having higher or lower level of message expression or stability from that of the template nucleic acid is produced.
 - 141. A method for modifying codons in a nucleic acid encoding a polypeptide with a xylanase activity to increase its expression in a host cell, the method comprising the following steps:

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- (a) providing a nucleic acid encoding a polypeptide with a xylanase activity comprising a sequence as set forth in claim 1 or claim 24; and,
- (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.
- 142. A method for modifying codons in a nucleic acid encoding a xylanase polypeptide, the method comprising the following steps:
 - (a) providing a nucleic acid encoding a polypeptide with a xylanase activity comprising a sequence as set forth in claim 1 or claim 24; and,
- (b) identifying a codon in the nucleic acid of step (a) and replacing it with a different codon encoding the same amino acid as the replaced codon, thereby modifying codons in a nucleic acid encoding a xylanase.

143. A method for modifying codons in a nucleic acid encoding a xylanase polypeptide to increase its expression in a host cell, the method comprising the following steps:

(a) providing a nucleic acid encoding a xylanase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and,

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- (b) identifying a non-preferred or a less preferred codon in the nucleic acid of step (a) and replacing it with a preferred or neutrally used codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in the host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to increase its expression in a host cell.
- 144. A method for modifying a codon in a nucleic acid encoding a polypeptide having a xylanase activity to decrease its expression in a host cell, the method comprising the following steps:
- (a) providing a nucleic acid encoding a xylanase polypeptide comprising a sequence as set forth in claim 1 or claim 24; and
- (b) identifying at least one preferred codon in the nucleic acid of step (a) and replacing it with a non-preferred or less preferred codon encoding the same amino acid as the replaced codon, wherein a preferred codon is a codon over-represented in coding sequences in genes in a host cell and a non-preferred or less preferred codon is a codon under-represented in coding sequences in genes in the host cell, thereby modifying the nucleic acid to decrease its expression in a host cell.
- 145. The method of claim 144, wherein the host cell is a bacterial cell, a fungal cell, an insect cell, a yeast cell, a plant cell or a mammalian cell.
 - 146. A method for producing a library of nucleic acids encoding a plurality of modified xylanase active sites or substrate binding sites, wherein the modified active sites or substrate binding sites are derived from a first nucleic acid comprising a sequence encoding a first active site or a first substrate binding site the method comprising the following steps:
 - (a) providing a first nucleic acid encoding a first active site or first substrate binding site, wherein the first nucleic acid sequence comprises a sequence that hybridizes

under stringent conditions to a sequence as set forth in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:65, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:75, SEQ ID NO:77, SEQ ID NO:79, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:85, SEQ ID NO:87, SEQ ID NO:89, SEQ ID NO:91, SEQ ID NO:93, SEQ ID NO:95, SEQ ID NO:97, SEQ ID NO:99, SEQ ID NO:101, SEQ ID NO:103, SEQ ID 10 NO:105, SEQ ID NO:107, SEQ ID NO:109, SEQ ID NO:111, SEQ ID NO:113, SEQ ID NO:115, SEQ ID NO:117, SEQ ID NO:119, SEQ ID NO:121, SEQ ID NO:123, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:129, SEQ ID NO:131, SEQ ID NO:133, SEQ ID NO:135, SEQ ID NO:137, SEQ ID NO:139, SEQ ID NO:141, SEQ ID NO:143, SEQ ID 15 NO:145, SEQ ID NO:147, SEQ ID NO:149, SEQ ID NO:151, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:157, SEQ ID NO:199, SEQ ID NO:161, SEQ ID NO:163, SEQ ID NO:165, SEQ ID NO:167, SEQ ID NO:169, SEQ ID NO:171, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:181, SEQ ID NO:183, SEQ ID NO:185, SEQ ID NO:187, SEQ ID NO:189, SEQ ID NO:191, SEQ ID NO:193, SEQ ID 20 NO:195, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, SEQ ID NO:203, SEQ ID NO:205, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:211, SEQ ID NO:213, SEQ ID NO:215, SEQ ID NO:217, SEQ ID NO:219, SEQ ID NO:221, SEQ ID NO:223, SEQ ID NO:225, SEQ ID NO:227, SEQ ID NO:229, SEQ ID NO:231, SEQ ID NO:233, SEQ ID NO:235, SEQ ID NO:237, SEQ ID NO:239, SEQ ID NO:241, SEQ ID NO:243, SEQ ID 25 NO:245, SEQ ID NO:247, SEQ ID NO:249, SEQ ID NO:251, SEQ ID NO:253, SEQ ID NO:255, SEQ ID NO:257, SEQ ID NO:259, SEQ ID NO:261, SEQ ID NO:263, SEQ ID NO:265, SEQ ID NO:267, SEQ ID NO:269, SEQ ID NO:271, SEQ ID NO:273, SEQ ID NO:275, SEQ ID NO:277, SEQ ID NO:279, SEQ ID NO:281, SEQ ID NO:283, SEQ ID NO:285, SEQ ID NO:287, SEQ ID NO:289, SEQ ID NO:291, SEQ ID NO:293, SEQ ID 30 NO:295, SEQ ID NO:297, SEQ ID NO:299, SEQ ID NO:301, SEQ ID NO:303, SEQ ID NO:305, SEQ ID NO:307, SEQ ID NO:309, SEQ ID NO:311, SEQ ID NO:313, SEQ ID NO:315, SEQ ID NO:317, SEQ ID NO:319, SEQ ID NO:321, SEQ ID NO:323, SEQ ID NO:325, SEQ ID NO:327, SEQ ID NO:329, SEQ ID NO:331, SEQ ID NO:333, SEQ ID NO:335, SEQ ID NO:337, SEQ ID NO:339, SEQ ID NO:341, SEQ ID NO:343, SEQ ID

NO:345, SEQ ID NO:347, SEQ ID NO:349, SEQ ID NO:351, SEQ ID NO:353, SEQ ID NO:355, SEQ ID NO:357, SEQ ID NO:359, SEQ ID NO:361, SEQ ID NO:363, SEQ ID NO:365, SEQ ID NO:367, SEQ ID NO:369, SEQ ID NO:371, SEQ ID NO:373, SEQ ID NO:375, SEQ ID NO:377 or SEQ ID NO:379, or a subsequence thereof, and the nucleic acid encodes a xylanase active site or a xylanase substrate binding site;

(b) providing a set of mutagenic oligonucleotides that encode naturallyoccurring amino acid variants at a plurality of targeted codons in the first nucleic acid; and,

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- (c) using the set of mutagenic oligonucleotides to generate a set of active site-encoding or substrate binding site-encoding variant nucleic acids encoding a range of amino acid variations at each amino acid codon that was mutagenized, thereby producing a library of nucleic acids encoding a plurality of modified xylanase active sites or substrate binding sites.
- 147. The method of claim 145, comprising mutagenizing the first nucleic acid of step (a) by a method comprising an optimized directed evolution system, gene site-saturation mutagenesis (GSSMTM), or a synthetic ligation reassembly (SLR).
 - 148. The method of claim 145, comprising mutagenizing the first nucleic acid of step (a) or variants by a method comprising error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, gene reassembly, gene site saturated mutagenesis (GSSMTM), synthetic ligation reassembly (SLR) and a combination thereof.
 - acid of step (a) or variants by a method comprising recombination, recursive sequence recombination, phosphothioate-modified DNA mutagenesis, uracil-containing template mutagenesis, gapped duplex mutagenesis, point mismatch repair mutagenesis, repair-deficient host strain mutagenesis, chemical mutagenesis, radiogenic mutagenesis, deletion mutagenesis, restriction-selection mutagenesis, restriction-purification mutagenesis, artificial gene synthesis, ensemble mutagenesis, chimeric nucleic acid multimer creation and a combination thereof.
 - 150. A method for making a small molecule comprising the following steps:

(a) providing a plurality of biosynthetic enzymes capable of synthesizing or modifying a small molecule, wherein one of the enzymes comprises a xylanase enzyme encoded by a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;

- (b) providing a substrate for at least one of the enzymes of step (a); and
- (c) reacting the substrate of step (b) with the enzymes under conditions that facilitate a plurality of biocatalytic reactions to generate a small molecule by a series of biocatalytic reactions.
- 151. A method for modifying a small molecule comprising the following 10 steps:
 - (a) providing a xylanase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 64, or a polypeptide encoded by a nucleic acid comprising a nucleic acid sequence as set forth in claim 1 or claim 24;
 - (b) providing a small molecule; and

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- 15 (c) reacting the enzyme of step (a) with the small molecule of step (b) under conditions that facilitate an enzymatic reaction catalyzed by the xylanase enzyme, thereby modifying a small molecule by a xylanase enzymatic reaction.
 - 152. The method of claim 151, comprising a plurality of small molecule substrates for the enzyme of step (a), thereby generating a library of modified small molecules produced by at least one enzymatic reaction catalyzed by the xylanase enzyme.
 - 153. The method of claim 151, further comprising a plurality of additional enzymes under conditions that facilitate a plurality of biocatalytic reactions by the enzymes to form a library of modified small molecules produced by the plurality of enzymatic reactions.
 - 154. The method of claim 153, further comprising the step of testing the library to determine if a particular modified small molecule which exhibits a desired activity is present within the library.
 - 155. The method of claim 154, wherein the step of testing the library further comprises the steps of systematically eliminating all but one of the biocatalytic reactions used to produce a portion of the plurality of the modified small molecules within the library by

testing the portion of the modified small molecule for the presence or absence of the particular modified small molecule with a desired activity, and identifying at least one specific biocatalytic reaction that produces the particular modified small molecule of desired activity.

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- 156. A method for determining a functional fragment of a xylanase enzyme comprising the steps of:
- (a) providing a xylanase enzyme, wherein the enzyme comprises a polypeptide as set forth in claim 64, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24; and
- (b) deleting a plurality of amino acid residues from the sequence of step (a) and testing the remaining subsequence for a xylanase activity, thereby determining a functional fragment of a xylanase enzyme.
- 15 157. The method of claim 156, wherein the xylanase activity is measured by providing a xylanase substrate and detecting a decrease in the amount of the substrate or an increase in the amount of a reaction product.
 - 158. A method for whole cell engineering of new or modified phenotypes by using real-time metabolic flux analysis, the method comprising the following steps:
 - (a) making a modified cell by modifying the genetic composition of a cell, wherein the genetic composition is modified by addition to the cell of a nucleic acid comprising a sequence as set forth in claim 1 or claim 24;
 - (b) culturing the modified cell to generate a plurality of modified cells;
 - (c) measuring at least one metabolic parameter of the cell by monitoring the cell culture of step (b) in real time; and,
 - (d) analyzing the data of step (c) to determine if the measured parameter differs from a comparable measurement in an unmodified cell under similar conditions, thereby identifying an engineered phenotype in the cell using real-time metabolic flux analysis.
 - 159. The method of claim 158, wherein the genetic composition of the cell is modified by a method comprising deletion of a sequence or modification of a sequence in the cell, or, knocking out the expression of a gene.

160. The method of claim 158, further comprising selecting a cell comprising a newly engineered phenotype.

- 161. The method of claim 160, further comprising culturing the selected cell, thereby generating a new cell strain comprising a newly engineered phenotype.
- 162. An isolated or recombinant signal sequence consisting of a sequence as set forth in residues 1 to 14, 1 to 15, 1 to 16, 1 to 17, 1 to 18, 1 to 19, 1 to 20, 1 to 21, 1 to 10 22, 1 to 23, 1 to 24, 1 to 25, 1 to 26, 1 to 27, 1 to 28, 1 to 28, 1 to 30, 1 to 31, 1 to 32, 1 to 33, 1 to 34, 1 to 35, 1 to 36, 1 to 37, 1 to 38, 1 to 40, 1 to 41, 1 to 42, 1 to 43 or 1 to 44, of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, 15 SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEO ID NO:60, SEO ID NO:62, SEO ID NO:64, SEO ID NO:66, SEO ID NO:68, SEQ ID NO:70, SEQ ID NO:72, SEQ ID NO:74, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:82, SEQ ID NO:84, SEQ ID NO:86, SEQ ID NO:88, SEQ ID 20 NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:98, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:106, SEQ ID NO:108, SEQ ID NO:110, SEQ ID NO:112, SEQ ID NO:114, SEQ ID NO:116, SEQ ID NO:118, SEQ ID NO:120, SEQ ID NO:122, SEQ ID NO:124, SEQ ID NO:126, SEQ ID NO:128, SEQ ID NO:130, SEQ ID NO:132; SEQ ID NO:134; SEQ ID NO:136; SEQ ID NO:138; SEQ ID NO:140; 25 SEQ ID NO:142; SEQ ID NO:144; NO:146, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:152, SEQ ID NO:154, SEQ ID NO:156, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:166, SEQ ID NO:168, SEQ ID NO:170, SEQ ID NO:172, SEQ ID NO:174, SEQ ID NO:176, SEQ ID NO:178, SEQ ID NO:180, SEQ ID NO:182, SEQ ID NO:184, SEQ ID NO:186, SEQ ID NO:188, SEQ ID NO:190, SEQ ID 30 NO:192, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:206, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:212, SEQ ID NO:214, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:220, SEQ ID NO:222, SEQ ID NO:224, SEQ ID NO:226, SEQ ID NO:228, SEQ ID NO:230, SEQ ID NO:232, SEQ ID NO:234, SEQ ID NO:236, SEQ ID NO:238, SEQ ID NO:240, SEQ ID

NO:242, SEQ ID NO:244, SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:252, SEQ ID NO:254, SEQ ID NO:256, SEQ ID NO:258, SEQ ID NO:260, SEQ ID NO:262, SEQ ID NO:264, SEQ ID NO:266, SEQ ID NO:268, SEQ ID NO:270, SEQ ID NO:272, SEQ ID NO:274, SEQ ID NO:276, SEQ ID NO:278, SEQ ID NO:280, SEQ ID NO:282, SEQ ID NO:284, SEQ ID NO:286, SEQ ID NO:288, SEQ ID NO:290, SEQ ID NO:292, SEQ ID NO:294, SEQ ID NO:296, SEQ ID NO:298, SEQ ID NO:300, SEQ ID NO:302, SEQ ID NO:304, SEQ ID NO:306, SEQ ID NO:308, SEQ ID NO:310, SEQ ID NO:312, SEQ ID NO:314, SEQ ID NO:316, SEQ ID NO:318, SEQ ID NO:320, SEQ ID NO:322, SEQ ID NO:324, SEQ ID NO:326, SEQ ID NO:328, SEQ ID NO:330, SEQ ID NO:332, SEQ ID NO:334, SEQ ID NO:336, SEQ ID NO:338, SEQ ID NO:340, SEQ ID 10 NO:342, SEQ ID NO:344, SEQ ID NO:346, SEQ ID NO:348, SEQ ID NO:350, SEQ ID NO:352, SEQ ID NO:354, SEQ ID NO:356, SEQ ID NO:358, SEQ ID NO:360, SEQ ID NO:362, SEQ ID NO:364, SEQ ID NO:366, SEQ ID NO:368, SEQ ID NO:370, SEQ ID NO:372, SEQ ID NO:374, SEQ ID NO:376, SEQ ID NO:378 or SEQ ID NO:380; or, consisting of a sequence as set forth in Table 4.

163. A chimeric polypeptide comprising at least a first domain comprising signal peptide (SP) having a sequence as set forth in claim 162, and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

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- The chimeric polypeptide of claim 163, wherein the heterologous 164. polypeptide or peptide is not a xylanase.
- 25 165. The chimeric polypeptide of claim 163, wherein the heterologous polypeptide or peptide is amino terminal to, carboxy terminal to or on both ends of the signal peptide (SP) or a xylanase catalytic domain (CD).
- 166. An isolated or recombinant nucleic acid encoding a chimeric 30 polypeptide, wherein the chimeric polypeptide comprises at least a first domain comprising signal peptide (SP) having a sequence as set forth in claim 162 and at least a second domain comprising a heterologous polypeptide or peptide, wherein the heterologous polypeptide or peptide is not naturally associated with the signal peptide (SP).

167. A method of increasing thermotolerance or thermostability of a xylanase polypeptide, the method comprising glycosylating a xylanase, wherein the polypeptide comprises at least thirty contiguous amino acids of a polypeptide as set forth in claim 60, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24, thereby increasing the thermotolerance or thermostability of the xylanase.

- 168. A method for overexpressing a recombinant xylanase in a cell comprising expressing a vector comprising a nucleic acid sequence as set forth in claim 1 or claim 24, wherein overexpression is effected by use of a high activity promoter, a dicistronic vector or by gene amplification of the vector.
 - 169. A method of making a transgenic plant comprising the following steps:
- (a) introducing a heterologous nucleic acid sequence into the cell, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24, thereby producing a transformed plant cell;
 - (b) producing a transgenic plant from the transformed cell.
- 170. The method as set forth in claim 169, wherein the step (a) further comprises introducing the heterologous nucleic acid sequence by electroporation or microinjection of plant cell protoplasts.
- 171. The method as set forth in claim 169, wherein the step (a) comprises introducing the heterologous nucleic acid sequence directly to plant tissue by DNA particle bombardment or by using an Agrobacterium tumefaciens host.

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- 172. A method of expressing a heterologous nucleic acid sequence in a plant cell comprising the following steps:
- (a) transforming the plant cell with a heterologous nucleic acid sequence operably linked to a promoter, wherein the heterologous nucleic sequence comprises a sequence as set forth in claim 1 or claim 24;
- (b) growing the plant under conditions wherein the heterologous nucleic acids sequence is expressed in the plant cell.

173. A method for hydrolyzing, breaking up or disrupting a xylancomprising composition comprising the following steps:

- (a) providing a polypeptide having a xylanase activity as set forth in claim 64, or a polypeptide encoded by a nucleic acid as set forth in claim 1 or claim 24;
 - (b) providing a composition comprising a xylan; and
- (c) contacting the polypeptide of step (a) with the composition of step (b) under conditions wherein the xylanase hydrolyzes, breaks up or disrupts the xylan-comprising composition.
- 174. The method as set forth in claim 173, wherein the composition comprises a plant cell, a bacterial cell, a yeast cell, an insect cell, or an animal cell.
 - 175. A dough or a bread product comprising a polypeptide as set forth in claim 64.

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176. A method of dough conditioning comprising contacting a dough or a bread product with at least one polypeptide as set forth in claim 64 under conditions sufficient for conditioning the dough.

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- 177. A beverage comprising a polypeptide as set forth in claim 64.
- 178. A method of beverage production comprising administration of at least one polypeptide as set forth in claim 64 to a beverage or a beverage precursor under conditions sufficient for decreasing the viscosity of the beverage.

- 179. The method of claim 178, wherein the beverage or beverage precursor is a wort or a beer.
- 180. A food, a feed or a nutritional supplement comprising a polypeptide as set forth in claim 64.
 - 181. A method for utilizing a xylanase as a nutritional supplement in an animal diet, the method comprising:

preparing a nutritional supplement containing a xylanase enzyme comprising at least thirty contiguous amino acids of a polypeptide as set forth in claim 64; and administering the nutritional supplement to an animal to increase utilization of a xylan contained in a feed or a food ingested by the animal.

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- 182. The method of claim 181, wherein the animal is a human.
- 183. The method of claim 181, wherein the animal is a human.
- 10 184. The method of claim 181, wherein the animal is a ruminant or a monogastric animal.
 - 185. The method of claim 181, wherein the xylanase enzyme is prepared by expression of a polynucleotide encoding the xylanase in an organism selected from the group consisting of a bacterium, a yeast, a plant, an insect, a fungus and an animal.
 - 186. The method of claim 185, wherein the organism is selected from the group consisting of an S. pombe, S. cerevisiae, Pichia pastoris, Pseudomonas sp., E. coli, Streptomyces sp., Bacillus sp. and Lactobacillus sp.

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- 187. An edible enzyme delivery matrix comprising a thermostable recombinant xylanase enzyme.
- 188. The edible enzyme delivery matrix of claim 187 comprising a polypeptide as set forth in claim 64.
 - 189. A method for delivering a xylanase supplement to an animal, the method comprising:

preparing an edible enzyme delivery matrix in the form of pellets comprising a granulate edible carrier and a thermostable recombinant xylanase enzyme, wherein the pellets readily disperse the xylanase enzyme contained therein into aqueous media, and administering the edible enzyme delivery matrix to the animal.

190. The method of claim 189, wherein the recombinant xylanase enzyme comprises a polypeptide as set forth in claim 64.

191. The method of claim 189, wherein the granulate edible carrier comprises a carrier selected from the group consisting of a grain germ, a grain germ that is spent of oil, a hay, an alfalfa, a timothy, a soy hull, a sunflower seed meal and a wheat midd.

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192. The method of claim 189, wherein the edible carrier comprises grain germ that is spent of oil.

193. The method of claim 189, wherein the xylanase enzyme is glycosylated to provide thermostability at pelletizing conditions.

- 194. The method of claim 189, wherein the delivery matrix is formed by pelletizing a mixture comprising a grain germ and a xylanase.
 - 195. The method of claim 189, wherein the pelletizing conditions include application of steam.
- 20 196. The method of claim 189, wherein the pelletizing conditions comprise application of a temperature in excess of about 80°C for about 5 minutes and the enzyme retains a specific activity of at least 350 to about 900 units per milligram of enzyme.
- 197. An isolated or recombinant nucleic acid comprising a sequence
 25 encoding a polypeptide having a xylanase activity and a signal sequence, wherein the nucleic acid comprises a sequence as set forth in claim 1.
 - 198. The isolated or recombinant nucleic acid of claim 197, wherein the signal sequence is derived from another xylanase or a non-xylanase enzyme.
 - 199. An isolated or recombinant nucleic acid comprising a sequence encoding a polypeptide having a xylanase activity, wherein the sequence does not contain a signal sequence and the nucleic acid comprises a sequence as set forth in claim 1.

200. An isolated or recombinant nucleic acid comprising a sequence as set forth in SEQ ID NO: 189, wherein SEQ ID NO: 189 contains one or more of the following mutations: the nucleotides at positions 22 to 24 are TTC, the nucleotides at positions 31 to 33 are CAC, the nucleotides at positions 34 to 36 are TTG, the nucleotides at positions 49 to 51 are ATA, the nucleotides at positions 31 to 33 are CAT, the nucleotides at positions 67 to 69 are ACG, the nucleotides at positions 178 to 180 are CAC, the nucleotides at positions 190 to 192 are TGT, the nucleotides at positions 190 to 192 are GTA, the nucleotides at positions 190 to 192 are GTG, the nucleotides at positions 190 to 192 are GTG, the nucleotides at positions 202 to 204 are GCT, the nucleotides at positions 235 to 237 are CCA, or the nucleotides at positions 235 to 237 are CCA, or the nucleotides at positions 235 to 237 are CCC.

201. A method for making a nucleic acid comprising a sequence as set forth in claim 200, wherein the mutations in SEQ ID NO: 189 are obtained by gene site saturated mutagenesis (GSSMTM).

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- 202. An isolated or recombinant polypeptide comprising an amino acid sequence comprising SEQ ID NO: 190, wherein SEQ ID NO: 190 contains one or more of the following mutations: the aspartic acid at amino acid position 8 is phenylalanine, the glutamine at amino acid position 11 is histidine, the asparagine at amino acid position 12 is leucine, the glycine at amino acid position 17 is isoleucine, the threonine at amino acid position 23 is threonine encoded by a codon other than the wild type codon, the glycine at amino acid position 60 is histidine, the proline at amino acid position 64 is cysteine, the proline at amino acid position 64 is valine, the serine at amino acid position 65 is valine, the glycine at amino acid position 68 is isoleucine, the glycine at amino acid position 68 is alanine, or the valine at amino acid position 79 is proline.
- 203. A method for reducing lignin in a wood or wood product comprising contacting the wood or wood product with a polypeptide as set forth in claim 64.
- 30 204. A detergent composition comprising a polypeptide as set forth in claim 64.
 - 205. A pharmaceutical composition comprising a polypeptide as set forth in claim 64.

206. A method for eliminating or protecting animals from a microorganism comprising a xylan comprising administering a polypeptide as set forth in claim 64.

- 207. The method of claim 206, wherein the microorganism is a bacterium.
 - 208. The method of claim 205, wherein the bacterium is a salmonellae.
- An isolated or recombinant nucleic acid comprising SEQ ID NO:189, 209. 10 wherein SEQ ID NO:189 comprises one or more or all of the following sequence variations: the nucleotides at positions 22 to 24 are TTC, the nucleotides at positions 22 to 24 are TTT, the nucleotides at positions 31 to 33 are CAC, the nucleotides at positions 31 to 33 are CAT, the nucleotides at positions 34 to 36 are TTG, the nucleotides at positions 34 to 36 are TTA, the nucleotides at positions 34 to 36 are CTC, the nucleotides at positions 34 to 36 are CTT, the nucleotides at positions 34 to 36 are CTA, the nucleotides at positions 34 to 36 are CTG, 15 the nucleotides at positions 49 to 51 are ATA, the nucleotides at positions 49 to 51 are ATT, the nucleotides at positions 49 to 51 are ATC, the nucleotides at positions 178 to 180 are CAC, the nucleotides at positions 178 to 180 are CAT, the nucleotides at positions 190 to 192 are TGT, the nucleotides at positions 190 to 192 are TGC, the nucleotides at positions 190 to 20 192 are GTA, the nucleotides at positions 190 to 192 are GTT, the nucleotides at positions 190 to 192 are GTC, the nucleotides at positions 190 to 192 are GTG, the nucleotides at positions 193 to 195 are GTG, the nucleotides at positions 193 to 195 are GTC, the nucleotides at positions 193 to 195 are GTA, the nucleotides at positions 193 to 195 are GTT, the nucleotides at positions 202 to 204 are ATA, the nucleotides at positions 202 to 204 are 25 ATT, the nucleotides at positions 202 to 204 are ATC, the nucleotides at positions 202 to 204 are GCT, the nucleotides at positions 202 to 204 are GCG, the nucleotides at positions 202 to 204 are GCC, the nucleotides at positions 202 to 204 are GCA, the nucleotides at positions 235 to 237 are CCA, the nucleotides at positions 235 to 237 are CCC, or the nucleotides at positions 235 to 237 are CCG.

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210. An isolated or recombinant polypeptide comprising an amino acid sequence comprising SEQ ID NO:190, wherein SEQ ID NO:190 comprises one or more or all of the following sequence variations: the aspartic acid at amino acid position 8 is phenylalanine, the glutamine at amino acid position 11 is histidine, the asparagine at amino

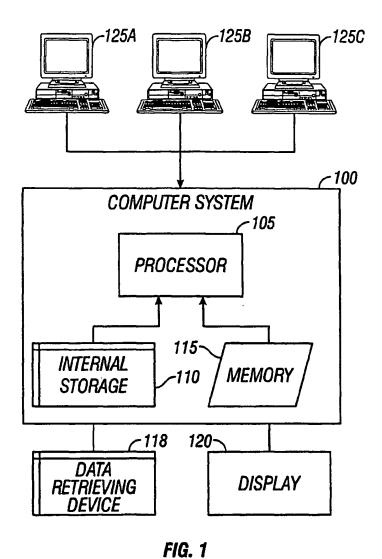
acid position 12 is leucine, the glycine at amino acid position 17 is isoleucine, the threonine at amino acid position 23 is threonine encoded by a codon other than the wild type codon, the glycine at amino acid position 60 is histidine, the proline at amino acid position 64 is cysteine, the proline at amino acid position 64 is valine, the serine at amino acid position 65 is valine, the glycine at amino acid position 68 is isoleucine, the glycine at amino acid position 68 is alanine, or the serine at amino acid position 79 is proline.

- 211. An isolated or recombinant nucleic acid comprising SEQ ID NO: 189, wherein SEQ ID NO:189 comprises one or more or all sequence variations set forth in Table 1 or Table 2.
- 212. An isolated or recombinant polypeptide encoded by the nucleic acid of claim 211.
- 213. An isolated or recombinant nucleic acid comprising SEQ ID NO:379, wherein SEQ ID NO:379 comprises one or more or all of the following sequence variations: the nucleotides at positions 22 to 24 are TTC, the nucleotides at positions 31 to 33 are CAC, the nucleotides at positions 49 to 51 are ATA, the nucleotides at positions 178 to 180 are CAC, the nucleotides at positions 193 to 195 are GTG, the nucleotides at positions 202 to 204 are GCT.
 - 214. An isolated or recombinant polypeptide comprising SEQ ID NO:380, wherein SEQ ID NO:380 comprises one or more or all of the following sequence variations: D8F, Q11H, G17I, G60H, S65V and/or G68A.
 - 215. The isolated or recombinant polypeptide of claim 210 or claim 214, wherein the polypeptide has a thermostable xylanase activity.

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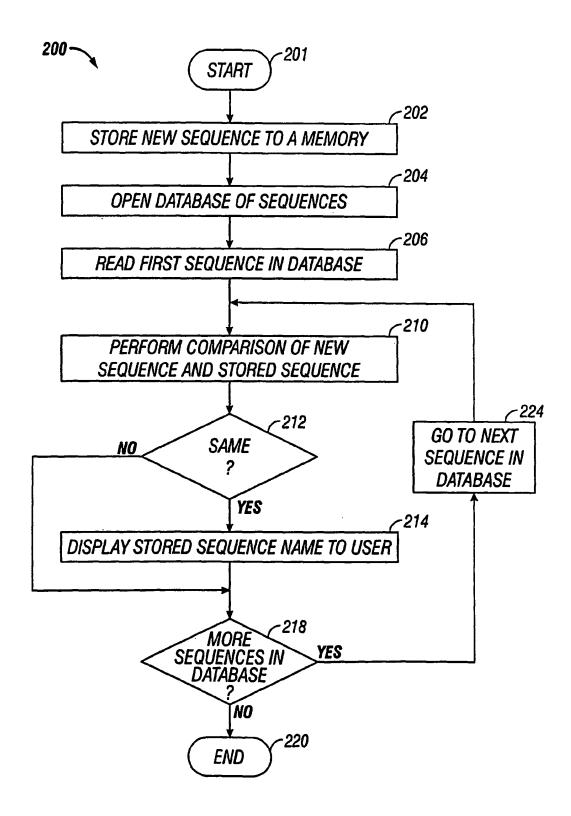
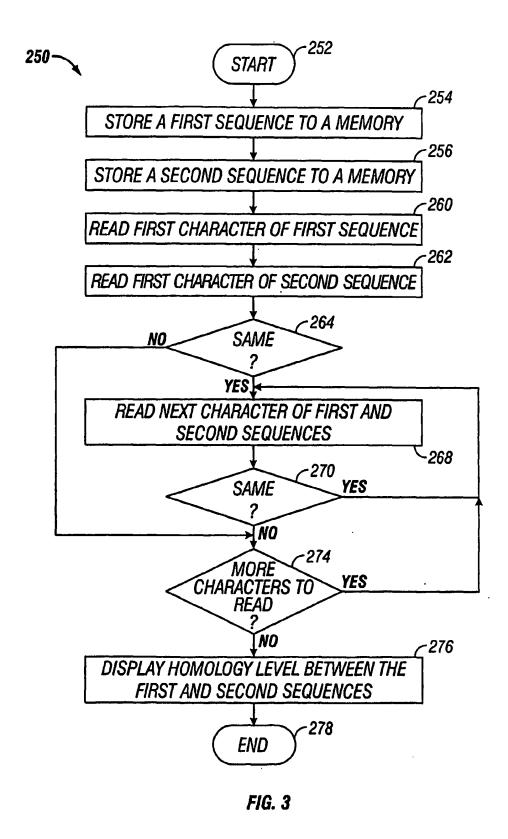


FIG. 2



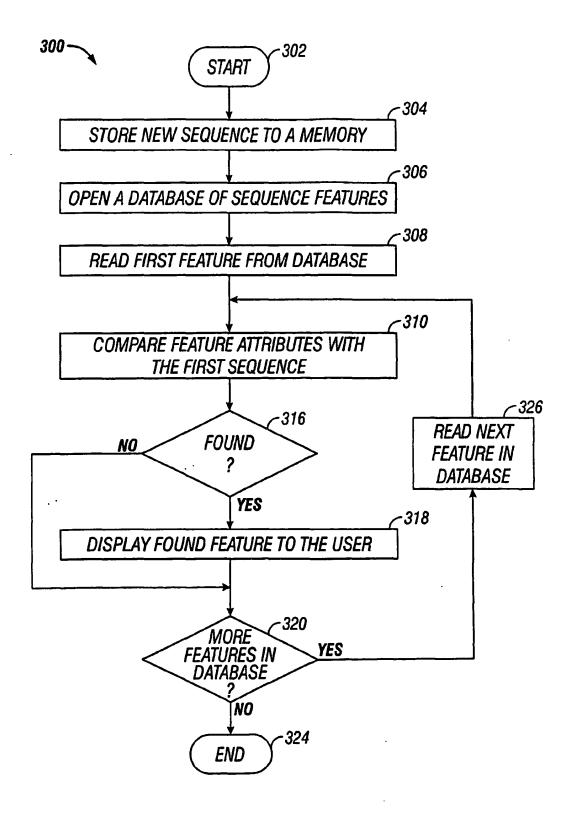


FIG. 4

Figure 5: Thermal Tolerance of Wild-type Xylanase (SEQ ID NOS:189 and 190) vs. 8x Mutant

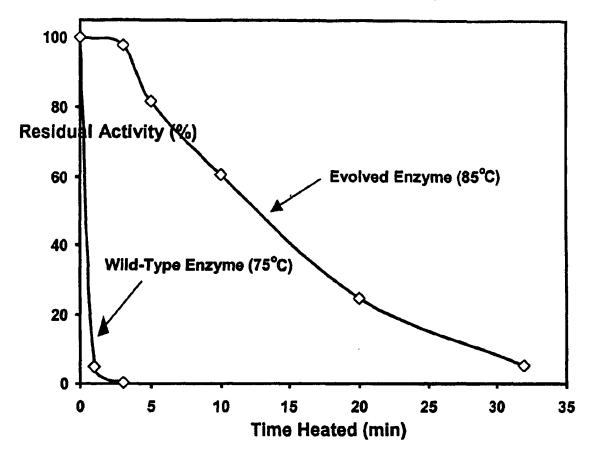


Figure 6

A **Amino Acid Sequence** T_ (°C) Mutant 1 17 60 64,65 68 8 11,12 61 67 70 66 67 67 69 66 64 Combine Mutations o...ocoecocecoco...ecoceecocecococec...c 9X 96

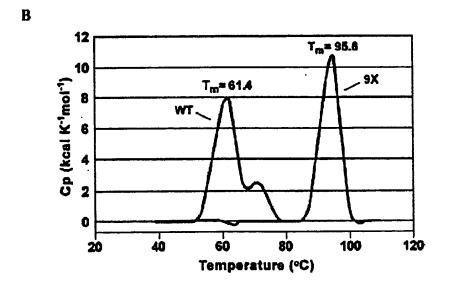


Figure 6

A - Amino Acid Sequence T. (°C) 79 189 Mutant 1 60 64,65 68 8 11,12 17 61 67 70 66 67 67 69 66 84 Combine Mutations 9X

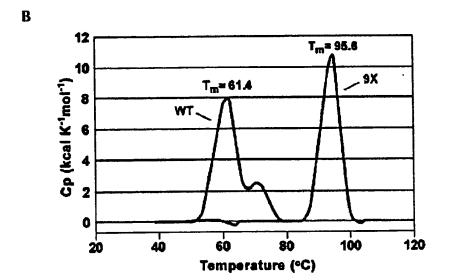


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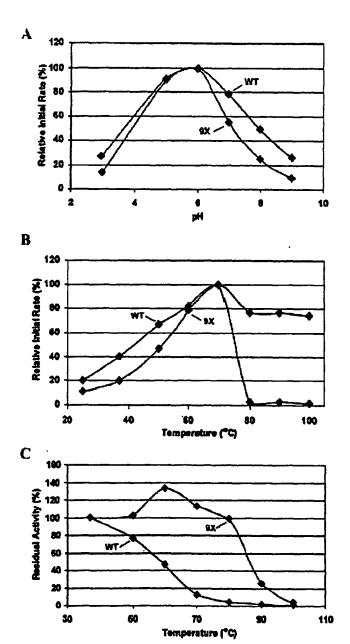


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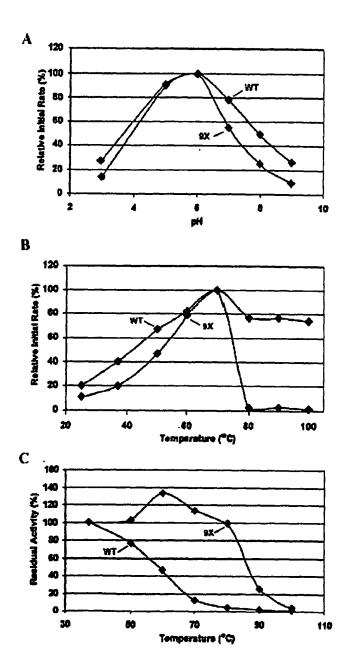


Figure 8

A **Amino Acid Sequence** T_m (°C) Varlant 11,12 17 60 64,86 79 189 61 6X-1 89 6X-2 90 7X-1 89 7X-2 91 7X-3 89 7X-4 81 8X-1 8X-2 90 8X-3 93 8X-4 94 9X

DEF ONH

G171

NIZL

GIGH PHAY GERA

96

37**9**P

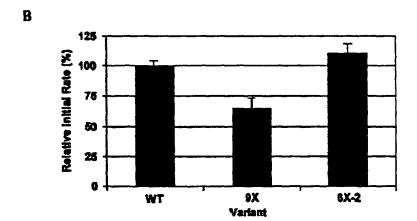
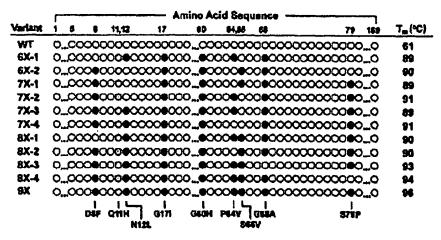


Figure 8

A



B

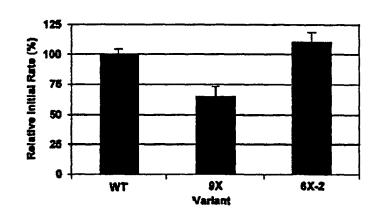


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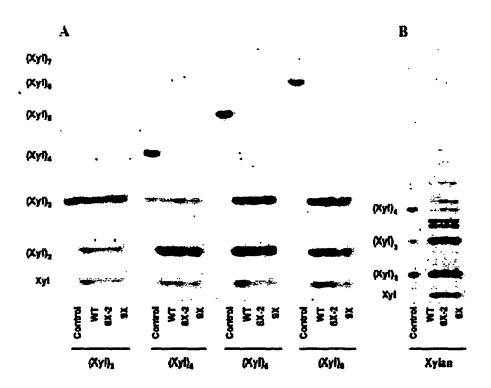


Figure 10

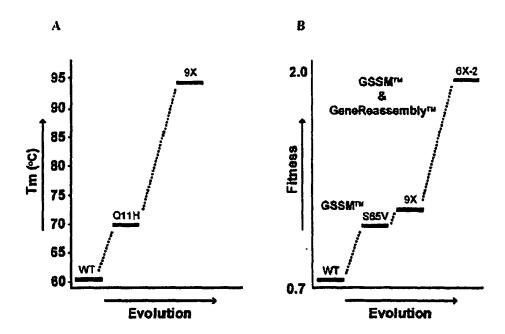


Figure 10

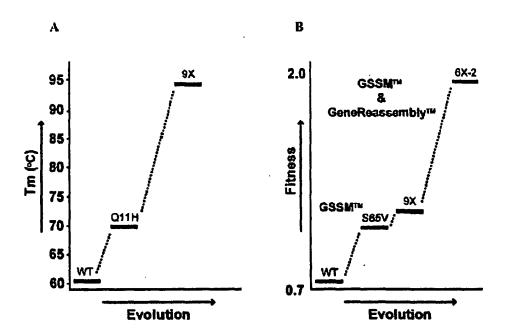
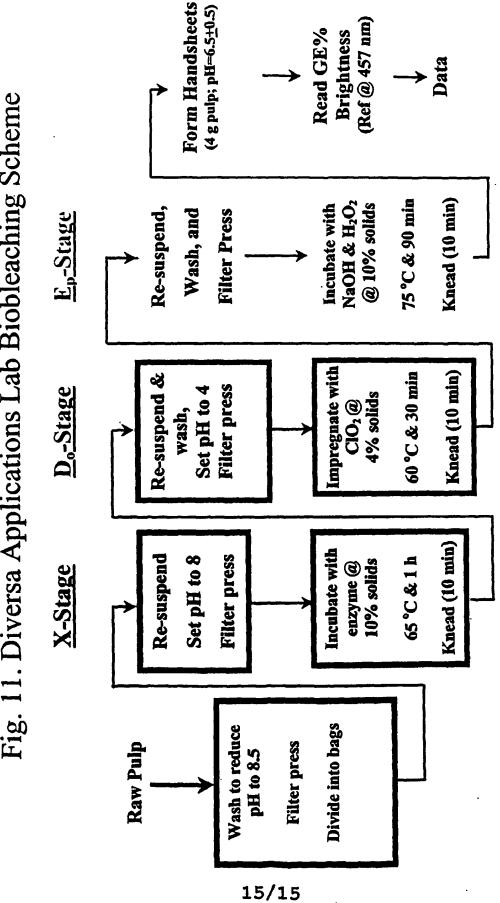


Fig. 11. Diversa Applications Lab Biobleaching Scheme



SEQUENCE LISTING

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Gly Ala Gln Ala Asp Ile Ala Val Thr Met Thr Leu Asn Gly Asn Thr
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Page 4

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145 150 155 160 Gln Leu Ala Ala Asn Pro Ala Ser Asp Gly Glu Val Trp Phe Val Met 165 170 175 Ala Leu Phe Phe Ala Asp Ala Arg Trp Gly Ser Gly Glu Gly Ile Tyr
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Phe Arg Tyr Asp Ala Phe Arg Val Gly Ala Asn Ile Gly Met Asp 305 315
Val Trp Phe His Pro Ser Glu Trp Tyr Arg Glu Gln Ala Asn Arg Gln
325 335
Leu Ser Phe Phe Ala Ser Gln Gly Ile Asp Asp Tyr Val Ala Glu Tyr
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Ser Leu Asp Gly Lys Pro Leu Ala Gly His Arg Ala Thr Gly Leu Ile
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Ala Thr Asn Ala Val Leu Ala Tyr Ala Ala Asp Pro Glu Ile Gly Gln 370 380
Pro Phe Val Gln Ala Leu Trp Asp Ala Glu Pro Pro Thr Gly Arg 385 390 395
Arg Tyr Tyr Asp Gly Leu Leu Tyr Met Met Gly Leu Leu Gln Ala Ser
Gly Asn Phe Arg Ile Tyr Glu Pro Gly Ile Thr Pro Arg Ala Glu Leu
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Pro Pro Pro Pro Arg Ala Ile Glu Gly Arg Phe Ala Pro Ile Thr
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Gly Arg Ala Leu Leu Leu Ile Gly Pro Asn Ala Asp Gly Val Asn Ala 450 455 460
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165 170 175 Ala Ser Asp Gly Glu Glu Trp Phe Val Met Ala Leu Leu Phe Ala Ala
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185
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<213> Unknown

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100 105 110 Ser Tyr Gly Met Met Ile Thr Val Gln Met Asp Lys Lys Ala Glu Phe Asp Ala Ile Trp Asn Trp Ala Lys Thr Tyr Met Tyr Gln Asp Ser Pro
130 140 Thr His Pro Ala Phe Gly Tyr Phe Ala Trp Ser Met Arg Arg Asp Gly 145 150 155 160 Val Ala Asn Asp Asp Met Pro Ala Pro Asp Gly Glu Glu Tyr Phe Val Thr Ala Leu Tyr Phe Ala Ala Ala Arg Trp Gly Asn Gly Glu Gly Ile 180 _ _ _ 185 _ 190 Phe Asn Tyr Gln Gln Glu Ala Asp Thr Ile Leu Ser Arg Met Arg His 195 200 205 Arg Gln Val Ile Thr Gly Pro Thr Asn Arg Gly Val Met Thr Ala Thr 210 215 220 Asn Leu Phe His Pro Glu Glu Ala Gln Val Arg Phe Thr Pro Asp Ile 225 230 235 240 Asn Asn Ala Asp His Thr Asp Ala Ser Tyr His Leu Pro Ser Phe Tyr 245 250 255 Glu Ile Trp Ala Arg Val Ala Pro Gln Glu Asp Arg Ala Phe Trp Ala 260 265 270 Lys Ala Ala Asp Val Ser Arg Asp Tyr Phe Ala Lys Ala Ala His Pro Val Thr Ala Leu Thr Pro Asp Tyr Gly Asn Phe Asp Gly Thr Pro Trp 290 295 300 Ala Ala Ser Trp Arg Pro Glu Ser Val Asp Phe Arg Tyr Asp Ala Trp 305 310 315 320 Arg Ser Val Met Asn Trp Ser Met Asp Tyr Ala Trp Trp Gly Lys Asp 325 330 335 Ser Gly Ala Pro Ala Arg Ser Asp Lys Leu Leu Ala Phe Glu Thr 340 345 350 Gln Glu Gly Lys Met Asn His Leu Tyr Ser Leu Asp Gly Lys Pro Leu 355 360 365 Gly Gly Gly Pro Thr Leu Gly Leu Ile Ser Met Asn Ala Thr Ala Ala 370 375 380 Met Ala Ala Thr Asp Pro Arg Trp His Asn Phe Val Glu Lys Leu Trp 385 390 395 400 Gln Gln Gln Pro Pro Thr Gly Gln Tyr Arg Tyr Tyr Asp Gly Val Leu 405 410 415 Tyr Leu Met Ala Leu Leu His Cys Ala Gly Glu Tyr Lys Ala Trp Ile 420 425 430 Pro Asp Gly Glu 435

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<211> 1224

<212> DNA

<213> Unknown

<220>

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agegeattga afgececaca attggateaa egetacaaaa aegagtteae gattggtgeg
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gcagtagaac cttatcaact acaaaatgaa aaagacgtac aaatgctaaa gcgccacttc
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aacagcattg ttgccgagaa cgtaatgaaa ccgatcagca ttcaacctga ggaaggaaaa
                                                                                                                360
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cgcttccata cactcgtttg gcacagccaa gtacctcaac ggttctttct tgacaaggaa
                                                                                                                420
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                                                                                                              1224
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20 25 30
Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu 35 40 45
Asp Gln Arg Tyr Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro
 Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys Arg His Phe 65 75 80
 Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro
85 90 95
Glu Glu Gly Lys Phe Asn Phe Glu Gln Ala Asp Arg Ile Val Lys Phe
100 105 110
Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp His 115 120 125
Ser Gln Val Pro Gln Arg Phe Phe Leu Asp Lys Glu Gly Lys Pro Met
130 140
Val Asn Glu Thr Asp Pro Val Lys Arg Glu Gln Asn Lys Gln Leu Leu
145 150 155 160
                                150
Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys
165 170 175
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195

Ile Lys Val Ala Phe Gln Ala Ala Arg Lys Tyr Gly Gly Asp Asn Ile
210

Lys Leu Tyr Met Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Thr
225

230

230

240

Asp Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp 180 185 190 Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln Ile Ala Gly Ile Asp Tyr 195 _ _ _ 200 205

Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu Gly Val Pro Ile 245 250 255

165

170

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Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu
                                            265
Ala Glu Ile Glu Lys Thr Ile Asn Met Phe Ala Ala Phe Gly Leu Asp
275 280 285
Asn Gln Ile Thr Glu Leu Asp Val Ser Met Tyr Gly Trp Pro Pro Arg
290 295 300
Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln 305 310 315 320
Ala Ala Arg Tyr Asp Arg Leu Phe Lys Leu Tyr Glu Lys Leu Ser Asp 325 330 335
Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp 340 345 350
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val
Val Asp Pro Asn Ala Pro Tyr Ala Lys Val Glu Lys Gly Lys Gly Lys
370 380
Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr 385 390 395 400
Trp Ala Ile Ile Asp His Lys
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                                                                                                  120
                                                                                                  180
gcgaccctgg cgaagggcga cctgctccac gctgcggtgg atttcacccg tgtggacgcg
ctgatgtact ttgcacggga caacgggatc cccatgcggt atcacaccct ggcctggcac
                                                                                                  240
                                                                                                  300
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                                                                                                  360
                                                                                                 420
                                                                                                  480
                                                                                                  540
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                                                                                                 780
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                                                                                                 840
caaaagctgg cgcagcttta cggcgattat ttcgccatgc tgaagaagct gaaggaggaa
                                                                                                 900
ggcgtčgača tčgaagccgt cačcttctgg ggcgtcaccg accaggačag čtggčtčacc
ggtttccgta aagagacaag ctatcccctc ctcttcgacc gcgccaagca ggccaaggat
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<211> 350
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<213> Unknown
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Ala Tyr Arg Ala Leu Ile Arg Arg His Tyr Asn Ser Leu Thr Ala Asp
Asn Gln Met Lys Pro Glu Ser Val Leu Asp Arg Thr Ala Thr Leu Ala 50 60
Lys Gly Asp Leu Leu His Ala Ala Val Asp Phe Thr Arg Val Asp Ala
65 75 80
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Leu Met Tyr Phe Ala Arg Asp Asn Gly Ile Pro Met Arg Tyr His Thr

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Leu Ala Trp His Asn Gln Thr Pro Arg Trp Phe Phe Ala Lys Asp Trp
100 105 110
Ser Asp Ala Glu Ser Ala Glu Pro Ala Ser Lys Glu Thr Met Leu Ala
115 120 125
Arg Leu Glu Asn Tyr Ile Leu Asp Val Met Asn His Val Asn Thr Lys
130 135 140
Phe Pro Gly Leu Val Tyr Thr Trp Asp Val Val Asn Glu Ala Ile Glu
145 150 155 160
Pro Glu Leu Lys Ala Pro Gly Leu Tyr Arg Thr Trp Ser Pro Trp Phe 165 170 175
Lys Thr Cys Gly Glu Asp Phe Leu Phe Thr Ala Phe Arg Ala Ala Arg
180
185
190
Lys Gly Gln Ala Pro Gly Gln Thr Leu Cys Tyr Asn Asp Tyr Asn Ala
195 200 205
Phe Glu Pro Val Lys Arg Asp Ala Ile Ile Asp Leu Leu Lys Lys Leu 210 220 220
Gln Ala Glu Asn Leu Val Asp Thr Met Gly Met Gln Gly His Tyr Val
225 230 235 240
Met Asp Trp Met Asn Ile Ser Leu Cys Glu Glu Ala Ala Arg Ala Tyr
245 250 255
Ala Ala Leu Gly Leu Lys Val Gln Val Thr Glu Leu Asp Ile His Cys 260 270
Asn Ser Asp Asp Glu Ala His Ser Gln Lys Leu Ala Gln Leu Tyr Gly 275 280 285
Asp Tyr Phe Ala Met Leu Lys Lys Leu Lys Glu Glu Gly Val Asp Ile
290 295 300
Glu Ala Val Thr Phe Trp Gly Val Thr Asp Gln Asp Ser Trp Leu Thr
305 310 315 320
Gly Phe Arg Lys Glu Thr Ser Tyr Pro Leu Leu Phe Asp Arg Ala Lys 325 330 335
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<212> DNA
<213> Unknown
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                                                                                                         120
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                                                                                                         240
                                                                                                         300
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aaacccgcga cacgggaaga acttctcaag cggatgcgcg atcacattca caaggtcgcc
ggccgataca agggtaaggt caagggctgg gacgtcgtca atgaggcgct ctccgacgga
ggtcaggaca ttctacgcga atctccgtgg cggcgaatca tcggagacga tttcatcgat
                                                                                                         420
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                                                                                                         720
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                                                                                                         780
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                                                                                                         840
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                                                                                                         960
                                                                                                       1020
cgcaccaatc acccgctact titcgatcgt gaactcaaac cgaagcccgt tcttccagtc
                                                                                                       1080
                                                                                                       1110
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<210> 16
<211> 369
<212> PRT
<213> Unknown
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<220>

<223> Obtained from an environmental sample

<221> SIGNAL <222> (1)...(20)

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100 105 110 Trp Val Phe Gln Gly Asp Asp Gly Lys Pro Ala Thr Arg Glu Glu Leu 115 120 125 Leu Lys Arg Met Arg Asp His Ile His Lys Val Ala Gly Arg Tyr Lys 130 135 140 Gly Lys Val Lys Gly Trp Asp Val Val Asn Glu Ala Leu Ser Asp 145 150 155 Gly Gln Asp Ile Leu Arg Glu Ser Pro Trp Arg Arg Ile Ile Gly Asp 165 170 175 Asp Phe Ile Asp His Ala Phe Arg Tyr Ala Arg Glu Ala Asp Pro Lys
180 185 190 Ala Glu Leu Tyr Tyr Asn Asp Tyr Asn Leu Glu Ile Pro Arg Lys Arg 195 200 205 Glu Asn Cys Ile Lys Leu Val Lys Gly Met Leu Glu Arg Gly Val Pro 215 Ile Asp Gly Ile Gly Thr Gln Ser His Phe Gln Leu Gly Phe Pro Ser 225 230 235 240 Leu Glu Asp Val Glu Thr Thr Ile Glu Glu Phe Gly Lys Leu Gly Leu 245 _ 250 _ 255 Lys Val Met Ile Thr Glu Leu Asp Val Asp Val Leu Pro Arg Asn Asn 260 265 270 Pro Gly Val Ala Asp Ile Ser Gln Arg Glu Gln Gly Ser Asn Pro Tyr 275 280 285 Thr Glu Gly Leu Pro Glu Asp Val Gln Lys Gln Leu Thr Lys Arg Tyr 290 295 300 Glu Asp Ile Phe Lys Ile Tyr Leu Lys His Gln Lys Thr Val Thr Arg 305 310 315 320 Val Thr Phe Trp Gly Leu Asp Asp Gly Gln Ser Trp Leu Asn Gly Phe 325 330 335 Pro Val Arg Gly Arg Thr Asn His Pro Leu Leu Phe Asp Arg Glu Leu 340 345 340 345 Lys Pro Lys Pro Val Leu Pro Val Leu Ile Glu Leu Gly Lys Lys 355 360 365 360 Arg

<210> 17 <211> 1035 <212> DNA <213> Bacteria

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aagtacttcg gctcggcac cgacaacccc gagttcaccg acgccgcta tctgaagctc 180
ctcggcagcg agttcgggca gaccaccccc ggcaacgcca tgaagtggta cgccaccgaa 240
cccgcgcccg gcgtcttcga cttcaccgcg ggcgacgagg tcgtggcctt cgccaaggcc 300
catcaccaga aggtccgcgg ccacaccctc gtctggcaca gccagctcc cgcctggctc 360
accgagcgca gctggaccg cgcggaactg cgcccgtcc tcaagaatca catccagaag 420
gtggcccggc actacaaggg caaggtcatc cactgggacg tcgtcaacga ggccttcaac 480
Page 13

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gaggacggca cctaccgcga gtcggtcttc tacaagacgc tcggccccgg ctacatcgcc
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                                                                               600
                                                                               660
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<210> 18 <211> 344 <212> PRT

<213> Bacteria

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100 105 110 His Ser Gln Leu Pro Ala Trp Leu Thr Glu Arg Ser Trp Thr Ala Ala 115 120 125 Glu Leu Arg Pro Val Leu Lys Asn His Ile Gln Lys Val Ala Arg His 130 _ _ 135 _ 140 _ _ Tyr Lys Gly Lys Val Ile His Trp Asp Val Val Asn Glu Ala Phe Asn
145
160 150 155 Glu Asp Gly Thr Tyr Arg Glu Ser Val Phe Tyr Lys Thr Leu Gly Pro 165 170 175 Gly Tyr Ile Ala Asp Ala Leu Arg Trp Ala His Glu Ala Asp Pro His 180 185 190 Ala Lys Leu Tyr Leu Asn Asp Tyr Asn Val Asp Gly Ile Gly Pro Lys 200 205 Ser Asp Ala Tyr Tyr Arg Leu Ile Lys Gln Leu Lys Ala Asp Gly Val Pro Val Glu Gly Phe Gly Ile Gln Gly His Leu Ala Leu Gln Tyr Gly 225 _ _ _ 230 _ _ 235 _ _ 240 Phe Pro Ala Asp Val Lys Gln Asn Met Gln Arg Phe Ala Asp Leu Gly 245 250 255 Thr Pro Ser Met Leu Ala Thr Gln Ala Thr Trp Tyr Ala Asp Tyr Val 275 280 285 Lys Ala Cys Leu Glu Val Arg Lys Cys Val Gly Val Thr Ile Trp Asp 290 295 300 Thr Asp Lys Tyr Ser Trp Ile Pro Ser Val Phe Pro Gly Glu Gly 310 315 Ala Ala Leu Pro Tyr Asp Glu Asn Leu Ala Pro Lys Pro Ala Tyr His 325 330 335 Ala Ile Arg Lys Val Leu Gly Gly 340

<210> 19 <211> 1152 <212> DNA

PCT/US03/19153

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100 105 110 His Thr Leu Île Trp His Ser Gln Thr Pro Ala Trp Phe Phe Val Asp 115 120 125

Lys Asn Gly Lys Glu Val Thr Arg Glu Val Leu Ile Glu Arg Met Arg 135 Lys His Ile Gln Thr Val Val Ser Arg Tyr Lys Gly Arg Val Phe Gly

145 150 155 160 Trp Asp Val Val Asn Glu Ala Ile Leu Asp Asn Gly Glu Trp Arg Lys
165 170 175 Ser Lys Phe Tyr Gln Ile Ile Gly Pro Gln Phe Ile Glu Leu Ala Phe
180
185
190 Lys Phe Ala His Asp Ala Asp Pro Asn Ala Glu Leu Tyr Tyr Asn Asp 195 200 205 Ser Thr Ala Ile Pro Glu Lys Arg Lys Gly Ile Met Arg Met Val 210 215 220 Gln Gln Val Lys Ala Ala Gly Gly Gln Val Thr Gly Ile Gly Met Gln 225 230 235 235 Page 15

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Glu His Asn Ala Leu Asp Asn Pro Pro Val Asp Glu Val Glu Lys Thr
                     245
                                                 250
Ile Leu Gly Phe Ala Ser Leu Gly Ala Lys Val Met Val Thr Glu Met
                                                                        270
                260
                                            265
Asp Ile Ser Val Leu Pro His Val Arg Pro Asn Met Gly Ala Glu Ile 275 280 285
Gly Glu Arg His Ala Tyr Ser Lys Ala Met Asn Pro Tyr Glu Lys Gly 290 295 300
Leu Pro Val Thr Lys Met Asn Glu Leu Gly Ala Arg Tyr Val Ala Phe 305 310 315 320
Phe Asn Leu Tyr Leu Lys His Arg Asp Lys Ile Ser Arg Val Thr Leu 325 330 335
Trp Gly Val Gly Asp Gly Asp Ser Trp Lys Asn Gly Trp Pro Ile Pro 340 350
Gly Arg Thr Asp Tyr Pro Leu Leu Phe Asp Arg Asn Tyr Gln Pro Lys
355 360 365
Pro Phe Val Lys Asp Ile Ile Ala Leu Thr Gln Lys Lys Lys 370 380
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<213> Unknown
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                                                                                                 180
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Ala Thr Leu Lys Asp Ala Phe Lys Asp Cys Phe Arg Ile Gly Val Ala
Leu Asn Gln Arg Gln Phe Thr Glu Gln Asp Thr Asn Gly Ala Thr Leu
50 55 60
Val Lys Arg Gln Phe Asn Ala Ile Ser Pro Glu Asn Val Met Lys Trp
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Ala Asn Ile His Pro Arg Pro Gly Pro Asp Gly Tyr Asn Phe Glu Ala
85 90 95
Ala Asp Arg Tyr Val Glu Phe Gly Glu Lys Asn Gly Met Phe Ile Val
Gly His Thr Leu Val Trp His Phe Gln Thr Pro Arg Trp Val Leu Gln
115 120 125
Gly Asp Gly Thr Asn Ala Ala Thr Arg Glu Leu Leu Gln Arg Met
130 140
Arg Asp His Ile His Thr Val Val Gly Arg Tyr Lys Gly Arg Ile Lys
145 150 155 160
Ala Trp Asp Val Val Asn Glu Ala Leu Asn Glu Asp Gly Thr Leu Arg
165 170 175
Arg Ser Gln Trp Tyr Arg Ile Ile Gly Glu Asp Tyr Ile Val Lys Ala
Phe Glu Tyr Ala His Glu Ala Asp Pro Ser Ala Glu Leu Arg Tyr Asn
195 200 205
Asp Tyr Ala Ile Glu Asn Glu Arg Lys Arg Asp Gly Val Ile Ala Leu 210 225 220

Val Lys Lys Leu Gln Ala Gln Lys Val Pro Leu Gly Gly Leu Gly Ser 225 230 240
Gln Thr His Ala Asn Leu Thr Trp Pro Asn Ala Glu Ser Leu Asp Thr
245 250 255
Ala Leu Thr Ala Phe Thr Glu Leu Gly Ile Pro Ile Ser Ile Thr Glu
260 265 270
Leu Asp Val Thr Ala Ser Gln Arg Gly Gln Leu Asn Gln Ser Ala Glu
275 280 285
Val Ser Gln Asn Gly Gln Ala Gly Glu Gly Gly Val Val Asp Gly Ala
290 295 300
Asn Gln Lys Leu Ala Glu Gln Tyr Ala Asn Phe Phe Arg Val Phe Leu 305 310 320
Lys His Arg Lys Asn Ile Glu Leu Val Thr Phe Trp Gly Val Thr Asp 325 330 335
Arg Asp Ser Trp Arg Arg Ile Gly Lys Pro Leu Leu Phe Asn Ala Glu
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Asn Val Asp Gln Met Ala Gly Lys Asp Ser Leu Ala Ile Glu Val Val 50 55 60
Lys Lys Asn Phe Ser Ser Ile Val Ala Glu Asn Cys Met Lys Met Glu 65 70 75 80
Asn Ile His Pro Val Lys Gly Glu Phe Phe Phe Asp Glu Ala Asp Ala
85 90 95
Tyr Val Glu Phe Gly Glu Lys Asn Asn Met Lys Ile Ile Gly His Thr
Leu Ile Trp His Ser Gln Ala Ala Lys Trp Ala Phe Val Asp Asp Glu
115 120 125
Gly Lys Asp Val Ser Arg Glu Glu Leu Ile Glu Arg Met Arg Asn His
130 135 140
Ile His Thr Ile Val Gly Arg Tyr Lys Gly Arg Val His Gly Trp Asp 155 150 155 160
Val Val Asn Glu Ala Ile Leu Asp Asn Gly Glu Trp Arg Gln Ser Lys
165 170 175
Trp Tyr Thr Ile Ile Gly Pro Glu Phe Val Gln Leu Ala Phe Glu Phe 180 185 190
Ala His Glu Ala Asp Pro Asn Ala Glu Leu Tyr Tyr Asn Asp Tyr Asn
195 200 205
Glu Trp Ile Pro Ala Lys Arg Asp Gly Ile Tyr Asn Met Val Lys Asp 210 220 220
Leu Ile Asp Lys Gly Val Lys Val Asp Gly Ile Gly Leu Gln Gly His 225 230 235 240
Ile Ala Leu Asp Ser Pro Ser Ile Glu Leu Tyr Glu Glu Ala Ile Val
245 250 255
Lys Tyr Ala Ser Leu Gly Val Gln Thr Met Val Thr Glu Leu Asp Ile
260 265 270
Thr Val Leu Pro Trp Pro Ser Gln Gln Val Thr Ala Asp Ile Ser Phe 275 280 285
Ser Ala Glu Leu Ser Thr Glu Tyr Asn Pro Phe Val Asn Gly Leu Pro
    290
                            295
Asp Ser Val Ser Val Glu Leu Thr Asn Arg Phe Ala Ser Phe Phe Glu
305 310 315 320
Leu Phe Leu Lys His Gln Asp Lys Ile Asp Arg Val Thr Leu Trp Gly
325 330 335
Val His Asp Gly Gln Ser Trp Lys Asn Asn Trp Pro Ile Arg Gly Arg 340 345 350
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                                                                                                  360
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Pro Leu Lys Pro Val Glu Gly Leu Lys Asp Ser Phe Lys Asp Lys Phe 35 40 45
Leu Met Gly Val Ala Leu Asn Lys Ala Gln Ile Leu Gly Arg Asp Thr
50 55 60
Leu Val His Ala Phe Thr Val Gln His Phe Asn Ser Ile Thr Ala Glu
65 70 75 80
Asn Glu Met Lys Trp Glu Arg Ile His Pro Gln Pro Asp Val Tyr Asp 85 90 95
Phe Thr Val Pro Asp Ser Leu Ile Ala Phe Gly Glu Arg Asn Gly Met
Phe Ile Val Gly His Thr Leu Val Trp His Ser Gln Val Pro Asp Trp
Val Phe Thr Asp Glu Lys Gly Lys Pro Leu Thr Arg Asp Ala Leu Leu
130 135 140
Gln Arg Met Lys Asp His Ile Tyr Ala Val Val Gly Arg Tyr Lys Gly
145 150 155 160
Lys Val Asp Gly Trp Asp Val Val Asn Glu Ala Leu Asp Glu Asp Gly 165 170 175
                Lys Ser Arg Trp His Glu Ile Ile Gly Asp Asp Tyr Ile
180 185 190
Gln Lys Ala Phe Glu Phe Thr Arg Glu Ala Asp Pro Gly Ala Glu Leu
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Val Gly Ile Gln Gly His Trp His Leu His Ser Pro Asp Leu Gln Glu
245 250 255
Ile Asp Ser Ser Leu Gln Ala Tyr Gly Gln Leu Gly Leu Lys Val Met
Ile Thr Glu Leu Asp Val Asn Val Ile Pro Glu Pro Ser Gly Ile Ile 275 280 285
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290 295 300
Trp Pro Glu Ser Phe Pro Asp Ser Met Gln Gln Val Leu Ala Ser Arg 305 310 315 320
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Tyr Ala Glu Leu Phe Gly Leu Phe Leu Lys His Ser Asp Lys Val Ser
Arg Val Thr Phe Trp Gly Ile His Asp Gly Tyr Ser Trp Lys Asn Asn 340 350
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435
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Val Asn Arg Glu Asp Val Gln Val Lys Lys Phe Val Gly Pro Gly Phe 515 520 525
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Lys Trp Tyr Ser Trp Ser Asp Thr Thr Asn Ser Gln Lys Thr Asn Thr 565 575
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585
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Lys Tyr Gly Thr Pro Val Ile Asp Gly Glu Ile Asp Glu Ile Trp Asn 595 600 605
Thr Thr Glu Glu Ile Glu Thr Lys Ala Val Ala Met Gly Ser Leu Asp
610 620 _
Lys Asn Ala Thr Ala Lys Val Arg Val Leu Trp Asp Glu Asn Tyr 625 630
Tyr Val Leu Ala Ile Val Lys Glu Pro Val Leu Asn Lys Asp Asn Ser
645 650 655
Asn Pro Trp Glu Gln Asp Ser Val Glu Ile Phe Val Asp Glu Asn Asn 660 665
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His Lys Thr Gly Tyr Tyr Glu Asp Asp Asp Ala Gln Phe Arg Val Asn 675 680 685
Lys Thr Ala Val Lys Leu Ile Glu Gly Gly Tyr Ile Val Glu Ala Ala
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Tie Lys Trp Lys Thr Ile Lys Pro Thr Pro Asn Thr Val Ile Gly Phe
725 730 735
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Asn Ile Gln Val Asn Asp Ala Asn Glu Lys Gly Gln Arg Val Gly Ile
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Val Tyr Ala Gly Lys Phe Asp Phe Gly Thr Ala Leu Pro Arg Asn Ala
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Phe Asn Asp Ile Gln Leu Leu Arg Leu Val Lys Asp Gln Phe Asn Ile
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Leu Thr Pro Glu Asn Glu Met Lys Pro Asp Ala Ile Leu Asp Val Tyr
Gly Ser Lys Leu Ala Glu Lys Asp Glu Thr Ala Val Ala Val Arg
Phe Glu Ala Cys Lys Thr Leu Leu Arg Phe Ala Gln Ser Asn Gly Leu
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Lys Val His Gly His Thr Leu Leu Trp His Asn Gln Thr Pro Glu Ala
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Leu Phe His Glu Gly Tyr Asp Thr Thr Lys Pro Met Ala Gly Arg Glu
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Val Met Leu Gly Arg Met Glu Asn Tyr Ile Arg Glu Val Leu Thr Trp
165 170 175
Thr Glu Glu Asn Tyr Pro Gly Val Ile Val Ser Trp Asp Val Val Asn 180 185 190
Glu Ala Ile Asp Asp Gly Thr Asn Gln Leu Arg Thr Gly Ala Asn Trp
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Tyr Lys Thr Val Gly Pro Asp Tyr Leu Ala Arg Ala Phe Glu Tyr Ala
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Arg Lys Tyr Ala Ala Glu Gly Val Leu Leu Tyr Tyr Asn Asp Tyr Asn 225 230 240
Thr Ala Tyr Gly Gly Lys Leu Tyr Gly Ile Val Asp Leu Leu Glu Ser
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Leu Ile Ala Glu Gly Asn Ile Asp Gly Tyr Gly Phe Gln Met His His 260 265 _ 270 _
Ser Leu Gly Glu Pro Ser Met Asp Met Ile Thr Arg Ala Val Glu Lys
275 280 285
Ile Ala Ser Leu Gly Leu Arg Leu Arg Val Ser Glu Leu Asp Ile Asn
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Ala Gly Lys Ala Thr Glu Lys Asn Phe Glu Ala Gln Lys Asn Lys Tyr
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240
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115
120
125 Ile Gly Leu Leu Arg Asp Ser Ile Met Thr Ile Val Gly Arg Tyr Lys 135 Gly Arg Ile Pro Ile Trp Asp Val Val Asn Glu Gly Ile Ala Asp Ser 145 150 155 155 Gly Gly Thr Leu Arg Asp Thr Pro Trp Arg Gln Leu Ile Gly Asp Asp 165 170 175 Tyr Ile Glu Leu Ala Phe Gln Phe Ala His Glu Ala Asp Pro Asp Ala 180 185 190 Leu Leu Phe Tyr Asn Asp Tyr Asn Thr Glu Gly Met Asn Pro Lys Ser Asp Ala Met Tyr Glu Met Val Ser Asp Phe Val Ala Arg Gly Ile Pro 210 215 220

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Gln Val Gln Phe Thr Glu Val Asp Ile Arg Tyr Ser Gly Glu Ala Thr
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Val Cys Leu Gly Asn Asp Ala Cys Thr Ala Phe Ile Thr Trp Gly Val
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Thr Asp Lys Tyr Thr Trp Leu Arg Gly Ala Asn Leu Gly Phe Tyr Asn 305 310 315 320
Asn Leu Ser Val Glu Pro Leu Leu Phe Asp Asp Asp Tyr Glu Pro Lys
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Pro Ala Tyr Phe Ala Val Leu Asp Ser Leu Ala Arg Arg Ala Gly Glu
340 345 350
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Glu Ala Pro Asp Ala Val Pro Gly Val Ile Tyr Tyr Ala Ala Tyr Pro 385 390 395
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Arg Gly Met Ile Asp Ser Gly Pro Thr Val Pro Gln Asp Asn Asp Thr
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Thr Met Thr Phe Ala Ala Ala Ala Asp Lys Thr Asn Leu Tyr Phe Leu
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Ile Met Ala Ala Asn Ile Asp Asn Asp Asn Pro Gly Ala Pro Ile Ile 500 505 510
Gly Gly Gly Asn Ser Asp Ile Ser Gln Val Lys Ala Ile Val Val Lys
515 _ 520 _ 525
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Val Trp Thr Ile Glu Pro Lys Gln Gly Ala Val Leu Gly Phe Gln Val
545 550 555 560
His Leu Asn Gly Ser Arg Thr Pro Asp Ala Asp Arg Asp Thr Lys Leu 565 570 575
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660

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50 60 Pro Glu Lys Glu Ile Pro Ala Leu Lys Glu Val Leu Lys Asp Tyr Phe 75 75 80 Lys Val Gly Val Ala Leu Pro Ser Lys Val Phe Leu Asn Pro Lys Asp 85 90 95 Ile Glu Leu Ile Thr Lys His Phe Asn Ser Ile Thr Ala Glu Asn Glu 100 105 _ _ 110 Met Lys Pro Asp Ser Leu Leu Ala Gly Ile Glu Asn Gly Lys Leu Lys 115 120 125 Phe Arg Phe Glu Thr Ala Asp Lys Tyr Ile Gln Phe Val Glu Glu Asn 130 140 Gly Met Val Ile Arg Gly His Thr Leu Val Trp His Asn Gln Thr Pro
145 150 160 Asp Trp Phe Phe Lys Asp Glu Asn Gly Asn Leu Leu Ser Lys Glu Ala 165 170 175 Asn Gln Pro Asp Gly Leu Arg Arg Ser Thr Trp Tyr Gln Ile Met Gly 210 220 Pro ASP Tyr Ile Glu Leu Ala Phe Lys Phe Ala Arg Glu Ala ASP Pro 235 236 240 Asp Ala Lys Leu Phe Tyr Asn Asp Tyr Asn Thr Phe Asp Pro Arg Lys Page 27

Arg Asp Ile Ile Tyr Asn Leu Val Lys Asp Leu Lys Glu Lys Gly Leu 260 265 270 Ile Asp Gly Ile Gly Met Gln Cys His Ile Ser Leu Ala Thr Asp Ile 275 280 285 Lys Gln Ile Glu Glu Ala Ile Lys Lys Phe Ser Thr Ile Pro Gly Ile 290 295 300 Glu Île His Île Thr Glu Leu Asp Met Ser Val Tyr Arg Asp Ser Ser 320 Ser Asn Tyr Pro Glu Ala Pro Arg Thr Ala Leu Ile Glu Gln Ala His Lys Met Met Gln Leu Phe Glu Ile Phe Lys Lys His Ser Asn Val Ile 340 Thr Asn val Thr Phe Trp Gly Leu Lys Asp Asp Tyr Ser Trp Arg Ala 355 Thr Arg Arg Asn Asp Trp Pro Leu Ile Phe Asp Lys Asp His Gln Ala 370 Met Asp Asp Ser Tyr Leu Met Ser Lys Pro Ile Glu Ile Leu Asp Glu 420 430 Glu Gly Asn Val Lys Ala Thr Ile Arg Ala Val Trp Lys Asp Ser Thr Asp Gly Val Ala Ile Phe Ile Asn Pro Asn Asn Glu Arg Thr Pro Tyr 480 Leu Gln Pro Asp Asp Thr Tyr Val Val Leu Trp Thr Asn Trp Lys Thr 495 Glu Val Asn Arg Glu Asp Val Gln Val Lys Lys Phe Val Gly Pro Gly 500 505 Phe Arg Arg Tyr Ser Phe Glu Met Ser Ile Thr Ile Pro Gly Val Glu Phe Lys Lys Asp Ser Tyr Ile Gly Phe Asp Val Ala Val Ile Asp Asp 530 535 540 Gly Lys Trp Tyr Ser Trp Ser Asp Thr Thr Asn Ser Gln Lys Thr Asn 560 Thr Met Asn Tyr Gly Thr Leu Lys Leu Glu Gly Ile Met Val Ala Thr 565 570 Ala Lys Tyr Gly Thr Pro Val Ile Asp Gly Glu Ile Asp Glu Ile Trp Asn Thr Thr Glu Glu Ile Glu Thr Lys Ala Val Ala Met Gly Ser Leu 595 600 605 Asp Lys Asn Ala Thr Ala Lys Val Arg Val Leu Trp Asp Glu Asn Tyr 610 620 Leu Tyr Val Leu Ala Ile Val Lys Asp Pro Val Leu Asn Lys Asp Asn 635 640 Ser Asn Pro Trp Glu Gln Asp Ser Val Glu Ile Phe Val Asp Glu Asn 655 Asn His Lys Thr Gly Tyr Tyr Glu Asp Asp Asp Ala Gln Phe Arg Val Asn Tyr Met Asn Glu Gln Thr Phe Gly Thr Gly Gly Ser Pro Ala Arg Phe Lys Thr Ala Val Lys Leu Ile Glu Gly Gly Tyr Ile Val Glu Ala 690 695 700 Ala île Lys Trp Lys Thr île Lys Pro Thr Pro Asn Thr Val île Gly 705 710 715 Phe Asn Ile Gln Val Asn Asp Ala Asn Glu Lys Gly Gln Arg Val Gly 735 Ile Ile Ser Trp Ser Asp Pro Thr Asn Asn Ser Trp Gln Asp Pro Ser 740 745 750 Lys Phe Gly Asn Leu Arg Leu Ile Lys 755 760

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145 150 155 160 Asn Gly Gly Ala Thr Gly Phe Ser Ala Arg Ile Ala Ala Thr Gly Ser 165 170 175 Gly Ser Ile Gln Val Ile Leu Gly Asn Leu Asn Asn Ala Pro Val Gly 180 185 190 Thr Leu Ala Val Ser Ser Thr Gly Asn Leu Gln Thr Trp Gln Asp Arg Ser Thr Ala Ile Ser Lys Val Thr Gly Val His Asp Val Tyr Leu Arg 210 215 220 Ala Thr Gly Asn Val His Val Gln Arg His Trp Phe Val Ala Ser Ala 225 230 235 240 Pro Ala Ala Ala Ser Ser Ser Gln Ala Ser Val Ser Ala Ser 245 250 255 Ser Gln Ala Ser Val Ser Ser Ser Gln Ala Ser Val Ala Ser Ser 260 265 270 Ser Ser Ser Arg Ala Ser Ser Ala Ser Ser Ser Val Ala Ala Gly
275 280 285 Gln Val Glu Val Gly Tyr Arg Leu Ser Ser Glu Trp Ala Ala Gly Phe 290 295 300 Cys Gly Val Val Thr Ile Arg Asn Pro Gly Ser Ser Pro Val Thr 305 310 315 Trp Ser Gly Ser Phe Asn Leu Pro Gly Gly Lys Ile Thr Gln Leu Trp 325 330 335 Asn Ala Asn Trp Thr Gln Asn Gly Ser Thr Val Thr Val Ser Ser Gln 340 345 350 Ala Trp Ser Gly Ala Ile Ala Ala Gly Ala Thr Ile Thr Thr Pro Gly 355 360 365 Phe Cys Ala Glu Arg Thr Ser Ser Asn Ala Ser Ser Ser Val Ala Ser 370 375 380 Ser Ser Val Ser Ser Ser Ser Ser Ala Ala Ala Ser Ser Ser 385 390 395 Ala Ala Ser Ser Val Pro Ser Thr Gly Ser Gly Gly Val Gly Ser Ser 410 415 Ala Ser Ser Ala Ser Ser Ala Ala Ala Pro Lys Gly Val Leu Glu Val 420 425 430 Gly Leu Ser Gly Leu Ser Ser Gln Ala Met Phe Ala Pro Leu Arg Val 440 445 435 Arg Thr Asp Ala Ala Ala Asn Lys Ala Tyr Val Glu Trp Pro Asn 455 460 Asn Gly Ala Asn Gln Ser Leu Ala Thr Pro Ala Asn Asp Ala Ala Gly 465 470 475 475 GÎN VAI GIU VAI Ala Phe VAI Leu Ala GIN Ala Ser Ala Vai Gin Phe 485 490 495 Asp Ile Glu Ala Asn Phe Ala Asn Ala Glu Asp Asp Ser Phe Tyr Phe 500 505 Gln Leu Asn Gly Gly Ala Trp Gln Thr Phe Asn Asn Ala Thr Thr Val 515 520 Gly <u>Trp</u> Gln Thr Leu Pro Val Ala Ser Leu Gly Asn Leu Ala Ala Gly 535 540 Arg His Val Leu Thr Leu Leu Arg Arg Glu Asp Gly Ala Lys Leu Gly 545 550 560 545 Lys Val Val Leu Ser Ala Ala Gln Ser Ser Ile Ser Arg Ala Thr Pro 565 570 575 Val Ala Tyr Ala Ser Pro Asn Asp Val Ala Asn Leu Phe 585 Ser Phe Pro Ile Gly Val Ala Val Ser Ala Gly Asn Glu Gly Asp Ser

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Leu His Pro Ala Glu Asn Thr Tyr Thr Phe Thr Gln Ala Asp Ala Leu
645 650 655
Ala Asp Tyr Ala Lys Ser Lys Gly Met Val Leu His Gly His Ala Leu 660 665 670
Val Trp His Ala Asp Tyr Gln Val Pro Asn Trp Met Lys Asn Tyr Thr
675 680 685
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690 700
     Tyr Ala Gly Lys Val Val Ser Trp Asp Val Val Asn Glu Ala Leu
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Ala Asp Gly Asn Ala Thr Ala Thr Lys Gly Phe Arg Ala Thr Asp Ser
Ile Phe Tyr Gln Lys Met Gly Ser Ser Phe Ile Glu Lys Ala Phe Ile 740 745 750
Ala Ala Arg Ala Ala Asp Pro Asn Ala Asp Leu Tyr Tyr Asn Asp Tyr
755 760 765
Gly Met Glu Gly Gly Asn Ser Lys Phe Asn Tyr Cys Met Ala Met Val
Asp Asp Phe Gln Lys Arg Gly Ile Pro Ile Asp Gly Ile Gly Phe Gln 785 790 795 800
Met His Ile Asn Ile Asp Trp Pro Ser Ser Ala Gln Ile Arg Ala Val
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Phe Ser Glu Val Val Lys Arg Gly Leu Lys Val Arg Ile Ser Glu Leu
820 825 830
Asp Ile Pro Val Asn Thr Thr Ala Gly Arg Phe Ala Ser Leu Asn Ala
835 840 845
Thr Ala Asn Glu Leu Gln Lys Lys Lys Tyr Arg Glu Val Val Ala Ala
850 855 860
Tyr Leu Asp Val Val Pro Pro Glu Leu Arg Gly Gly Ile Thr Val Trp
865 870 886
Gly Leu Ser Asp Asn Gly Ser Trp Leu Val Thr Pro Thr Lys Pro Asp
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                                                                                                      960
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                                                                                                     1080
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1140 1143

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65 70 75 80 Met Lys Ser Met Tyr Leu Gln Pro Glu Glu Gly Lys Phe Phe Asp 85 90 95 Asp Ala Asp Lys Phe Val Asp Phe Gly Leu Gln Asn Asn Met Phe Ile Ile Gly His Cys Leu Ile Trp His Ser Gln Ala Pro Lys Trp Phe Phe 115 120 125 Thr Asp Glu Asn Gly Asn Thr Val Ser Pro Glu Val Leu Lys Gln Arg
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Asn Gly Ser Leu Cys Gly Thr Ala Thr Thr Ser Ser Val Arg Ser Ser 130

Val Ala Ala Thr Ser Ser Ser Arg Ser Ser Val Ala Pro Ser Ser Ile 155

150

150

160
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Pro Ala Ser Ser Thr Pro Arg Ser Ser Thr Pro Ala Thr Ser Ser Ser 170 165 Ala Ser Ser Phe Ser Val Pro Ala Asn Asn Phe Ala Gln Asn Gly Gly 190 180 185 Val Glu Ser Gly Leu Thr Asn Trp Gly Thr Thr Ala Gly Thr Val Thr 195 200 205 Arg Ser Thr Ala Asp Lys His Ser Gly Thr Ala Ser Ala Leu Ile Thr 210 215 220 Gly Arg Thr Ala Ala Trp Asn Gly Leu Thr Phe Asn Val Gly Ala Leu 225 230 235 240 Thr Asn Gly Asn Gln Tyr Gln Val Asn Val Trp Val Lys Leu Ala Pro
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420
425
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PCT/US03/19153

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                                                                                       840
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aagatattga gagaatatag tcatgtaatc agttcgatta ctttttgggg agctgcagat gattatactt ggttagatga ttttcctgtc aaaggaagaa aaaactggcc atttgtttt
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                                                                                      1011
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20 25 30
Asp Ser Gln Gln Asp Leu Leu Arg Lys His Phe Asn Ser Ile Thr Ala
Glu Asn Glu Met Lys Phe Glu Glu Leu Gln Pro Glu Pro Gly His Phe 50 55 60
Thr Phe Gly Val Ala Asp Glu Ile Val Ser Phe Ala Lys Glu Asn Gly 65 70 75 80
Met Lys Val Arg Gly His Thr Leu Val Trp His Asn Gln Thr Pro Asp 90 95
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Trp Met Phe Leu Asn Glu Asp Gly Ser Val Thr Asp Arg Glu Thr Leu 100 105 110 Leu Glu Arg Met Lys Leu His Ile Thr Thr Val Met Gln His Tyr Lys
115
120
125 Gly Gln Ala Tyr Cys Trp Asp Val Val Asn Glu Val Ile Ala Asp Glu 130 140 Gly Thr Glu Leu Phe Arg Lys Ser Lys Trp Thr Glu Ile Ile Gly Asp 150 155 160 Asp Phe Val Glu Lys Ala Phe Glu Tyr Ala His Glu Ala Asp Pro Glu 165 170 175 Ala Leu Leu Phe Tyr Asn Asp Tyr Asn Glu Ser His Pro Asn Lys Arg 180 185 190 Glu Lys Ile Phe Thr Leu Val Lys Gly Leu Val Asp Lys Gly Ile Pro 195 200 205 Ile His Gly Ile Gly Leu Gln Ala His Trp Asn Leu Thr Gly Pro Ser Tyr Glu Asp Ile Arg Ala Ala Leu Glu Lys Tyr Ala Thr Leu Gly Leu 225 230 235 240 Glu Ile His Leu Thr Glu Leu Asp Val Ser Val Phe Asn Tyr Glu Asp 245 250 255 Arg Arg Thr Asp Leu Thr Glu Pro Thr Lys Asp Met Gln Ala Leu Gln 260 265 270 Ala Glu Arg Tyr Thr Glu Leu Phe Lys Ile Leu Arg Glu Tyr Ser His Val lle Ser Ser lle Thr Phe Trp Gly Ala Ala Asp Asp Tyr Thr Trp 290 295 300 Leu Asp Asp Phe Pro Val Lys Gly Arg Lys Asn Trp Pro Phe Val Phe 305 310 315 320 Asp Glu Asn Gln Glu Pro Lys Glu Ser Phe Trp Asn Ile Ile Asp Phe

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 Leu Asn Thr Gln Ile Val Asp Gly Lys Asp Pro Lys Leu Thr Ala Leu 50 60 _____
 The Thr Lys Glu Phe Asn Ser Ile Thr Ala Glu Asn Cys Gln Lys Trp 80 75
 Glu Arg Leu Arg Asn Glu Lys Asp Gly Ser Trp Glu Trp Lys Asp Ser
 Asp Ala Phe Val Asn Phe Gly Val Ala His Asn Met His Ile Val Gly 100

His Thr Leu Gly Trp His Ser Gln Ile Pro Asp Ser Val Phe Lys Asn 120

125
 Lys Asp Gly Ser Tyr Ile Ser Lys Glu Ala Leu Ala Lys Lys Gln Gln
130
135
140
 Glu His Ile Thr Thr Leu Val Asp Arg Tyr Lys Gly Lys Ile Ala Ala
150 155 160
 Ser His Trp Tyr Asn Ile Met Gly Asp Asp Phe Leu Val Asn Ala Phe 180
Lys Leu Ala His Glu Thr Asp Pro Lys Ala His Leu Met Tyr Asn Asp 200
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Lys Arg Leu Leu Lys Leu Gly Ala Pro Ile His Gly Leu Gly Met Gln
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Ala His Ile Gly Leu Asp Ala Asp Met Lys Asn Phe Glu Asp Ser Ile 245 250 255
Val Ala Tyr Ser Glu Leu Gly Leu Arg Ile His Leu Thr Glu Leu Asp
260 265 270
Ile Asp Val Leu Pro Ser Val Trp Asn Leu Pro Val Ala Glu Val Ser
275 280 285
Thr Arg Phe Glu Tyr Lys Pro Glu Arg Asp Pro Tyr Ile Lys Gly Leu 290 295 300
Pro Lys Glu Ile Asp Glu Lys Leu Ala Lys Ala Tyr Glu Ser Leu Phe 305 310 315 320
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Gly Val Ser Asp Asp Ala Ser Trp Leu Asn Gly Phe Pro Ile Pro Gly 340 345 350
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                                                                                                        300
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                                                                                                        420
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aagaaccata tttatacggt cgtggggcgt tacaaaggcc gtgtccacgg ctgggatgtg
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                                                                                                      1020
                                                                                                      1080
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Leu Lys Asp Ala Leu Ser Gly Lys Phe Tyr Ile Gly Ala Ala Leu Asn
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Tie Gln Arg Thr Glu Gly Glu Phe Asp Phe Ser Leu Ala Asp Gln Phe 85 90 95
Val Ala Phe Gly Glu Lys His Asn Met His Ile Val Gly His Thr Leu
100 105 110
Ile Trp His Ser Gln Ala Pro Arg Trp Phe Phe Thr Gly Ala Asp Gly
Asn Glu Val Ser Arg Glu Val Leu Ile Glu Arg Met Lys Asn His Ile
130 135 140
Tyr Thr Val Val Gly Arg Tyr Lys Gly Arg Val His Gly Trp Asp Val 145 150 155 160
Val Asn Glu Ala Ile Glu Asp Asn Gly Ser Trp Arg Asn Ser Lys Phe 165 170 175
Tyr Gln Ile Leu Gly Asp Glu Phe Val Glu Leu Ala Phe Lys Phe Ala
180 185 190
Ala Glu Ala Asp Pro Asp Ala Glu Leu Tyr Tyr Asn Asp Tyr Ser Met 200 205
Ala Leu Glu Gly Arg Arg Asn Gly Val Ile Arg Met Val Lys Asn Leu
210 215 220 _____
Gln Ser Lys Gly Leu Lys Ile Asp Gly Ile Gly Met Gln Gly His Leu
225 230 235 240
Leu Met Asp Ser Pro Thr Leu Glu Ala Tyr Glu Glu Ser Ile Leu Ala
245 250 255
Tyr Ser Gly Leu Gly Val Lys Val Met Ile Thr Glu Leu Asp Leu Ser
Ala Leu Pro Trp Pro Ala Arg Gln Gln Gly Ala Asp Ile Ala Leu Arg
Ala Glu Tyr Glu Ala Arg Met Asn Pro Tyr Thr Glu Gly Leu Thr Asp
290 295 300
 Ser Ala Ser Val Ala Trp Asn Gln Arg Met Gly Asp Phe Phe Ser Leu 320
 Phe Leu Lys His Gln Asp Lys Ile Ser Arg Val Thr Leu Trp Gly Val
 Thr Asp Asn Gln Ser Trp Lys Asn Asn Phe Pro Met Arg Gly Arg Thr 340 345 350
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260 265 270
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Page 46

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85 90 95 Met Ile Ser Leu Glu Lys Val Ala Asp Gly Ile Thr Gly Trp Glu Asn 100 105 110 Leu Val Arg Gly Thr Val Lys Lys Gly Glu Trp Thr Thr Leu Ser Gly 120 125 Thr Tyr Thr Phe Ala Asp Tyr Glu Ser Tyr Val Leu Tyr Val Glu Thr 130 135 140 Ser Asp Ala Pro Thr Leu Asp Phe Glu Ile Arg Asn Phe Arg Val Glu 145 150 150 160 Ser Pro Asn Gly Ile Pro Glu Pro Lys Ala Thr Glu Ala Pro Ala Val 165 170 175 Val Ser Glu Ala Thr Asp Ile Pro Ser Leu Lys Asp Ala Tyr Ala Asp 180 185 190 Tyr Phe Asp Phe Gly Ala Ala Val Pro Gln Ser Ala Phe Thr Ser Arg 195 200 205 Asp Asn Ile Gln Leu Met Glu Leu Met Lys Asn Gln Phe Ser Ile Leu 210 225 220 Thr Pro Glu Asn Glu Leu Lys Pro Asp Ser Val Leu Asp Val Ser Ala 225 230 240 Ser Lys Gln Leu Ala Lys Glu Asp Glu Thr Ala Val Val Arg Phe 245 250 255 Asn Gly Ala Lys Ser Leu Leu Arg Phe Ala Gln Gln Asn Gly Ile Lys
260 265 270 Val His Gly His Val Leu Val Trp His Ser Gln Thr Pro Glu Ala Phe 275 280 285 Phe His Glu Gly Tyr Asp Pro Lys Asn Pro Leu Val Ser Arg Glu Val 290 295 300 Met Leu Gly Arg Leu Glu Asn Tyr Ile Arg Glu Val Leu Thr Gln Thr 305 310 315 320 Glu Glu Leu Tyr Pro Gly Val Ile Val Ser Trp Asp Val Val Asn Glu
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460
455 Ala Met Met Glu Leu Met Leu Arg Phe Ala Asp Gln Thr Glu Ala Val 465 470 480 Gln val Trp Gly Ile Thr Asp Thr Met Ser Trp Arg Ser Ser Ser Tyr Page 47

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                                                                                                            960
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Gln Pro Asp Ile Thr Glu Ile Ile Lys Arg Asp Phe Ser Ser Leu Thr 50 60
Pro Glu Asn Ala Met Lys Trp Glu Ser Val Arg Thr Ala Asp Gly Gly 65 70 75 80
Trp Lys Trp Ala Asp Ala Asp Gln Phe Val Thr Phe Ala Thr Glu His
Lys Ile His Ala Val Gly His Thr Leu Ala Trp His Ser Gln Ile Pro
100 105 110
Asp Ser Val Phe Lys Asn Glu Lys Gly Glu Tyr Ile Lys Ser Thr Glu
115 120 125
Leu Ser Lys Lys Met Glu Glu His Ile Thr Thr Ile Val Gly Arg Tyr
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Lys Gly Lys Leu Asp Ala Trp Asp Val Val Asp Glu Ala Val Gly Asp 145 150 160
Asp Asn Gln Met Arg Lys Ser His Tyr Tyr Asn Ile Leu Gly Glu Asp
165 170 175
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His Leu Met Tyr Asn Asp Tyr Asn Ile Glu Lys Asp Gly Lys Arg Glu
195 200 205
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His Gly Leu Gly Ile Gln Gly His Ile Ala Val Asp Gly Pro Ser Ile
225 230 235 240
Ala Asp Ile Glu Lys Ser Ile Leu Ala Tyr Ala Asp Leu Gly Leu Arg
Val His Phe Thr Glu Leu Asp Ile Asp Val Leu Pro Gln Ile Trp Asn 260 265 270
Leu Pro Val Ala Glu Ile Ser Thr Arg Phe Glu Tyr Lys Pro Glu Arg
275 280 285
Asp Pro Phe Lys Asn Gly Leu Ser Lys Glu Met Asn Asp Lys Leu Ser 290 295 300
Ala Arg Tyr Glu Glu Leu Phe Thr Leu Phe Ile Lys His Lys Asp Lys 305 310 315 320
lle Asp Arg Ile Thr Leu Trp Gly Val Ser Asp Asp Ala Thr Trp Leu 325 330 335
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gacagtőgaa gcgatctgct tcgttcctcc ccgtggcttg cgtcgatcgg ggaggatttt
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Glu Met Lys Phe Glu Arg Leu His Pro Ser Glu Glu Val Tyr Thr Phe

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Glu Gln Ala Asp Gln Ile Val Ser Phe Ala Lys Ser Asn Gly Met Ser
65 70 75 80
Val Arg Gly His Thr Leu Val Trp His Asn Gln Thr Pro Glu Trp Val
85 90 _ 95
Phe Gln Asp Ser Ser Gly Gly Thr Ala Gly Arg Glu Leu Leu Leu Ala
Arg Met Lys Ser His Ile Asp Glu Val Val Gly Arg Tyr Arg Gly Asp
115 120 125
Ile Tyr Ala Trp Asp Val Val Asn Glu Ala Ile Ala Asp Ser Gly Ser
130 135 140
Asp Leu Leu Arg Ser Ser Pro Trp Leu Ala Ser Ile Gly Glu Asp Phe
145 150 155 160
lle Ala Lys Ala Phe Glu Tyr Ala His Glu Ala Asp Pro Gln Ala Leu
165 170 175
Leu Phe Tyr Asn Asp Tyr Asn Glu Ser Val Pro Glu Lys Arg Glu Lys
180 185
Ile Tyr Thr Leu Leu Lys Ser Leu Lys Glu Gln Asp Val Pro Ile His
195 200 205
Gly Val Gly Leu Gln Ala His Trp Asn Leu Glu Phe Pro Ser Leu Asp
210 215 220
Asp Ile Arg Arg Ala Ile Glu Arg Tyr Ala Ser Leu Gly Met Ile Leu
225 _____ 230 ____ 235 ____ 240
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Thr Asp Leu Ala Ala Pro Thr Glu Glu Met Leu Glu Arg Gln Ala Glu
260 265 270
Arg Tyr Gly Gln Leu Phe Arg Leu Leu Lys Glu Tyr Ser Gly Ser Val 275 280 285
Thr Ser Val Thr Phe Trp Gly Ala Ala Asp Asp Tyr Thr Trp Leu Asp 290 295 300
His Phe Pro Val Arg Gly Arg Lys Asn Trp Pro Phe Val Phe Asp Glu
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20 25 30
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Lys Arg Ala Val Ala Ile Ile Arg Asn Gln Phe Ser Ser Ile Val Ala
50 55 60
Glu Asn Cys Met Lys Ser Met Phe Leu Gln Pro Gln Glu Gly Lys Phe 65 70 75 80
Phe Phe Asp Asp Ala Asp Lys Phe Val Asp Phe Gly Met Lys Asn Asn 85 90 95
Met Phe Val Ile Gly His Thr Leu Ile Trp His Ser Gln Leu Pro Lys 100 105 110
Trp Phe Phe Thr Asp Lys Asn Gly Lys Asp Val Ser Pro Glu Val Leu
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Lys Gln Arg Met Lys Asn His Ile Thr Thr Val Val Ser Arg Tyr Lys
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Gly Lys Val Lys Gly Trp Asp Val Val Asn Glu Ala Ile Leu Glu Asp
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Gly Thr Tyr Arg Lys Ser Lys Phe Tyr Glu Ile Leu Gly Glu Asp Phe
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Ile Pro Leu Ala Phe Gln Tyr Ala Gln Glu Ala Asp Pro Asn Ala Glu
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Leu Tyr Tyr Asn Asp Tyr Asn Glu Trp Tyr Pro Glu Lys Val Lys Ala
195 200 205
Val Ile Thr Met Val Glu Lys Leu Lys Ser Arg Gly Ile Arg Ile Asp
210 215 220
Gly Val Gly Met Gln Ala His Val Gly Met Asp Ile Pro Ser Ile Asn
225 230 240
Glu Tyr Glu Lys Ala Ile Leu Ala Tyr Ser Asn Ala Gly Val Lys Val
245 250 255
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Ser Ala Asn Val Ser Asp Thr Val Ala Tyr Gln Lys Glu Met Asn Pro
275 280 285
Tyr Thr Lys Gly Leu Pro Asn Glu Val Glu Ala Lys Trp Glu Lys Arg
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Tyr Leu Asp Phe Phe Ser Leu Phe Leu Lys His Lys Asp Lys Ile Arg 305 310 315 320
Arg Val Thr Leu Trp Gly Val Thr Asp Lys Gln Ser Trp Lys Asn Asp 325 330 335
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Asn
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Phe Val Ala Gly Thr Asp Gly Trp Tyr Ala Arg Gly Ala Gln Lys Val
Tyr Arg Thr Thr Glu Glu Thr Leu Arg Thr Glu Gly Arg Thr Ser Asp 50 60
Trp His Ser Pro Gly Arg Asp Phe Asp Leu Val Glu Gly Gly Val Tyr 65 70 75 80 Val Leu Ser Val Glu Val Phe Gln Asp Glu Ala Asp Asn Ala Ser Phe 90 95
Met Ile Ser Ile Ala His Ser Lys Asp Gly Thr Glu Thr Tyr Glu Asn
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Leu Ala Arg Gly Thr Ala Lys Arg Gly Glu Trp Val Thr Leu Thr Gly
115
120
125
Thr Tyr Thr Ala Gly Asn Phe Asp Arg Asn Val Leu Tyr Val Glu Thr
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Thr Gly Ser Pro Glu Leu Ser Tyr Glu Ile Arg Asn Phe Arg Val Glu
145 150 155 160
Ala Pro Asn Gly Val Pro Glu Pro Lys Ala Thr Glu Pro Pro Met Val
Ile Glu Ala Val Glu Asn Leu Pro Gly Leu Lys Asn Ala Tyr Ala Gly
180

Lys Phe Asp Phe Gly Ala Ala Val Pro Gly Tyr Ala Phe Gly Asp Pro
195
200
205
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Asn Glu Leu Lys Pro Asp Ala Val Leu Asp Val Ala Ala Ser Lys Arg 225 230 230 240

Leu Ala Gln Glu Asp Glu Thr Ala Val Ala Val His Phe Asp Gly Ala

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                                      280
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290 295 300
                                 295
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305 310 315
Tyr Pro Gly Val Ile Val Ser Trp Asp Val Val Asn Glu Ala Ile Asp 325 330 335
Asp Gly Thr Asn Trp Leu Arg Asn Ser Asn Trp Tyr Lys Thr Val Gly 340 345 350
Gly Asp Phe Val Asn Arg Ala Phe Glu Phe Ala Arg Met Tyr Ala Ala 355 360 365
Asp Gly Val Leu Leu Tyr Tyr Asn Asp Tyr Asn Thr Ala Tyr Pro Ala 370 380
Lys Arg Lys Gly Ile Ile Lys Leu Leu Gly Gln Leu Ile Glu Glu Gly 385 390 395 400
Asn Ile Asp Gly Tyr Gly Phe Gln Met His His Ser Thr Gly Glu Pro
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Asp Gln Arg Tyr Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro
Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys Arg His Phe 65 70 75 80
Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro
85 90 95
Glu Glu Gly Lys Phe Asn Phe Glu Gln Ala Asp Arg Ile Val Lys Phe
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Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp His 115 120 125
Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met 130 140
Val Asn Glu Thr Asp Pro Val Lys Arg Glu Gln Asn Lys Gln Leu Leu
145 150 155 160
Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys
165 170 175
Asp Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp 180 185 190
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195 200 205
Ile Lys Val Ala Phe Gln Thr Ala Arg Lys Tyr Gly Gly Asn Lys Ile
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230 235 240
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Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu
Ala Glu Ile Glu Lys Thr Ile Asn Met Phe Ala Ala Leu Gly Leu Asp
275 280 285
Asn Gln Ile Thr Glu Leu Asp Val Ser Met Tyr Gly Trp Pro Pro Arg 290 295 300
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Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp 340 345 350
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Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val 355 360 365
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Met Thr Arg His Ala Phe Gly Phe Gly Thr Ala Val Ser Phe Gly Leu 65 70 75 80
Val Val Gly Ser Gly Tyr Asn Pro Thr Tyr Arg Ala Lys Leu Glu Asp
85 90 95
Leu Thr Gly Asp Gly Arg Thr Phe Asn Met Ala Thr Pro Glu Asn Glu
Leu Lys Trp Pro Ala Trp Glu Ser Glu Trp Pro Ile Ser Asn Arg Arg
Lys Ile Asp Val Ile Asn Trp Leu Arg Ala Lys Gly Tyr Ser Ile Arg
130 135 140
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155

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Glu Gln Asn Arg Asn Asn Pro Gln Tyr Ile Tyr Asp Arg Val Arg Asn 165 170 175

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Asp Val Phe Arg Trp Ala Lys Ala Ala Asp Ser Thr Ala Arg Leu Tyr
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Ile Asn Glu Tyr Asn Ile Ile Asn Asn Tyr Ala Asn Glu Gln Pro Thr
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Arg Asn Tyr Tyr Lys Trp Ile Ile Ala Arg Leu Ile Ser Lys Gly Ala 260 265 270
Pro Ile Glu Gly Ile Gly Ile Gln Gly His Ile Ser Ala Pro Leu Pro 275 280 285
Ser Met Ser Glu Val Lys Ala Ala Leu Asp Glu Met Ala Val Phe Gly
290 295 300
Leu Pro Leu Ala Ile Thr Glu Tyr Asp Val Thr Gly Val Ser Glu Glu 305 310 315 320
Val Glu Ala Asn Phe Met Arg Asp Phe Leu Thr Met Val Phe Ser His
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Pro Ala Val Glu Ser Phe Val Met Trp Gly Phe Trp Ser Gly Ala His 340 345
Trp Arg Asp Asn Ala Pro Leu Phe Arg Ala Asp Trp Ser Leu Lys Pro
355 360 365
Ser Gly Gln Val Phe Leu Asp Leu Val Phe Arg Arg Trp Trp Thr Asp 370 380
Thr Thr Gly Val Thr Gly Pro Asp Gly Ser Trp Ser Val Arg Gly Phe 385 390 395 400
Leu Gly Asp Tyr Val Val Glu Val Gln Val Gly Glu Val Ser Val Thr
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                                                 410
Lys Ser Leu Arg Leu Glu Ser Pro Gln Asp Thr Thr Thr Leu Glu Val
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Val Val Ser Ser Val Lys Val Gly Glu Lys Pro Thr Glu Asp Val Leu
435 440 445
Arg Val Gln Gly Phe Gly Pro Asp Pro Phe Val Glu Gly Thr Ala Leu 450 455 460
Arg Tyr Trp Leu Gly Arg Pro Ala Asp Val Glu Leu Ala Val Tyr Asp 465 470 475 480
Val Leu Gly Arg Gln Val Tyr Ala Val Gln Lys His Arg Val Ala Gly
Trp His Thr Glu Trp Val Glu Ala Ser His Trp Pro Ala Gly Leu Tyr 500 505 510
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Lys Ile Gln
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1020

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410
415
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Asp Asn Pro Thr Asn Gln Trp Thr Asp Thr Ser Val Glu Thr Phe Lys
50
55
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Gln Thr Met Gln Tyr Leu Asn Asp Ser Gly Tyr Asn Thr Leu Ser Ala 65 70 80
Glu Gln Tyr Val Lys Ile Met Asp Gly Thr Ala Thr Ala Pro Glu Lys
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95
Pro Ile Leu Leu Thr Phe Asp Asp Gly Thr Pro Glu Phe Ile Thr Asn 100 105 110
Ala Leu Pro Val Leu Lys Gln Tyr Asn Met Lys Ala Val Leu Phe Ile
115 120 125
Val Ser Asp Trp Ile Gly Gly Gly Phe Ser Met Ser Lys Glu Gln Leu
130 135 140
Gln Ser Leu Ala Asn Glu Pro Ser Leu Ser Leu Glu Asn His Thr Lys
145 150 155 160
Thr His Asp Gly Thr Ile Trp Gly Thr Asn Gly Gly Val Arg Ser Thr
Ile Thr Lys Glu Gln Ala Glu Asp Gln Ile Ile Ser Ala Asn Thr Tyr
180 185 190
Leu Lys Ser Ile Thr Gly Lys Asp Pro Val Leu Met Ala Tyr Pro Tyr
195 200 205
Gly Ser Tyr Asn Asp Ile Ala Lys Leu Val Asn Gln Glu Asn Gly Ile 210 _____ 220
    Tyr Ala Phe Lys Val Gly Tyr Pro Asn Glu Asp Asn Tyr Ala Met 230 235 240
Gly Arg His Tyr Val Thr Asn Gln Ser Val Ala Gln Ile Ala Gln Met
Ile Gly Gly Pro Val Pro Glu Pro Thr Pro Glu Pro Gly Asn Gln Thr
Glu Thr Val Tyr Gln Glu Thr Phe Ala Ser Asp Ile Gly Val Ala Val
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Gln Ala Gly Asn Pro Gln Val Thr His Val Ser Gly Met Val Phe Ala
290 295 300
Gly Asn Asp Asp Gly Lys Ala Ile Ser Val Ser Gly Arg Thr Asn 305 310 315
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420
425
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445 Ser Ala Lys Leu Thr Pro Val Ser Glu Leu Val Phe Glu Gly Asn Ser Asp Gly Lys Ala Ile Ser Val Asn Gly Arg Ser Asn Asn Trp Asp Gly 465 470 475 480 Val Asp Ile Pro Phe Ser Ser Val Ser Met Gln Asn Gly Lys Ala Tyr 485 490 495 Thr Ile Thr Val Thr Gly Phe Val Tyr Ser Ser Val Ser Val Pro Glu
500 505 510 Gly Ala Gln Ala Leu Leu Gln Asn Val Asp Ser Tyr Asn Gly Leu Tyr
515 520 525 Ala Ala Asp Val Lys Ala Gly Gln Thr Phe Thr Leu Thr Gly Gln
530 540 Tyr Thr Val Asp Thr Ser Lys Asp Arg Ala Leu Arg Ile Gln Ser Asn 545 550 560 Asp Ala Gly Lys Thr Val Pro Phe Tyr Ile Gly Asp Ile Leu Ile Thr 565 570 575 Glu Lys Ala Ala Ser Gly Gly Gly Gly Asp Asp Gly Arg Leu Pro Ala Glu Pro Phe Thr Ala Ile Asn Phe Glu Asp Gln Asn Met Gly Gly Phe 600 Glu Gly Arg Ala Gly Thr Glu Thr Leu Thr Val Thr Asn Glu Ala Asn 610 615 620 His Thr Asp Gly Gly Ser Tyr Ala Leu Lys Val Glu Gly Arg Ser Gln 625 630 635 640 Ala Trp His Gly Pro Ala Leu His Val Glu Lys Tyr Val Asp Lys Asp 645 655 Ser Glu Tyr Lys Ile Ser Ala Trp Val Lys Leu Ile Ser Pro Ala Thr 660 665 670 Ser Gln Leu Gln Leu Ser Thr Gln Val Gly Asn Gly Gly Thr Ala Ser Tyr Asn Asn Leu Gln Gly Lys Thr Ile Ser Thr Glu Asp Gly Trp Val Lys Leu Glu Gly Thr Tyr Arg Tyr Ser Ser Val Gly Asp Glu Phe Leu 705 710 715 720 Thr Ile Tyr Val Glu Ser Ser Asn Asn Ser Thr Ala Ser Phe Tyr Ile 725 730 735 Asp Asp Ile Thr Phe Glu Ser Thr Gly Ser Gly Pro Ile Glu Val Glu 740 745 750 Asp Leu Thr Pro Ile Lys Asp Val Tyr Gln Asp Asp Phe Leu Ile Gly 755 760 765 Asn Ala Val Ser Ala Ser Asp Leu Glu Gly Asn Arg Leu Lys Leu Leu 770 780 Asn Met His His Asn Val Val Thr Ala Glu Asn Ala Met Lys Pro Asp 785 790 795 800 Gln Ala Tyr Asn Ala Glu Lys Gln Phe Asp Phe Thr Asp Glu Asn Ala 805 810 815 Leu Val Asp Lys Val Leu Asp Gln Gly Leu Gln Leu His Gly His Val Leu Val Trp His Gln Gln Thr Pro Glu Trp Leu Phe Thr Ala Glu Asn 835 840 845 Gly Ala Pro Leu Ser Arg Glu Ala Ala Leu Ala Asn Leu Arg Thr His 850 855 860 Val Lys Thr Val Val Glu Asn Tyr Gly Asn Lys Val Ile Ser Trp Asp Page 60

865 870 Val Val Asn Glu Ala Ile Ile Asp Asn Pro Pro Asn Pro Thr Asp Trp 885 890 895 Lys Ala Ser Leu Arg Lys Ser Gly Trp Tyr Lys Ser Ile Gly Pro Asp 900 905 910 Phe Val Glu Gln Ser Phe Leu Ala Ala Lys Glu Val Leu Asn Glu Lys 915 920 925 Gly Leu Asn Ile Lys Leu Tyr Tyr Asn Asp Tyr Asn Asp Asp Asn Gln 930 935 940 Ser Lys Ala Glu Ala Ile Tyr Gln Met Val Lys Asp Ile Asn Glu Lys 945 950 955 960 Tyr Ala Lys Glu His Asp Gly Asp Leu Leu Ile Asp Gly Ile Gly Met
965 970 975 Gln Ala His Tyr Asn Lys Asn Thr Asn Pro Glu Asn Val Lys Leu Ser 980 985 990 Leu Glu Lys Phe Ile Thr Leu Gly Val Glu Val Ser Val Thr Glu Leu 995 1000 1005 Asp Ile Thr Ala Gly Thr Asn Asn Val Leu Thr Glu Lys Glu Ala Ile 1010 1015 1020 Ala Gln Gly Tyr Leu Tyr Ala Gln Leu Phe Lys Ile Tyr Lys Glu His 1025 1030 1035 1040 1040 Ala Glu His Ile Ser Arg Val Thr Phe Trp Gly Leu Asn Asp Ala Thr Ser Trp Arg Ala Ala Gln Ser Pro Leu Leu Phe Asp Lys Asp Leu Gln 1060 1065 1070 Ala Lys Pro Ala Tyr Tyr Ala Val Ile Asp Pro Asp Thr Phe Thr Val Glu Asn Gln Pro Glu Val Arg Glu Ala Asn Gln Gly Ser Ala Val Ser 1090 1095 1100 Gly Thr Pro Val Ile Asp Gly Thr Val Asp Gly Val Trp Ser Asn Ala 1105 1110 1115 1120 1120 Thr Glu Leu Pro Ile Asn Arg Phe Gln Met Ala Trp Gln Gly Ala Asn 1125 1130 1135 Gly Val Ser Lys Val Leu Trp Asp Asn Glu Asn Leu Tyr Val Leu Ile 1140 1145 1150 Gln Val Ser Asp Ser Gln Leu Asp Lys Ser Ser Pro Asn Pro Trp Glu 1155 1160 1165 Gln Asp Ser Ile Glu Val Phe Val Asp Glu Asn Asn Ala Lys Thr Ser 1170 1175 1180 Ser Phe Glu Asp Gly Asp Gly Gln Tyr Arg Val Asn Phe Asp Asn Glu 1185 1190 1195 1200 Thr Ser Phe Asn Pro Val Arg Val Gly Glu Gly Phe Glu Ser Ala Thr 1205 1210 1215 Lys Ala Ser Gly Asn Gly Tyr Thr Val Glu Val Lys Ile Pro Phe Lys
1220

Thr Ile Thr Pro Asn Asn Asn The Thr Thr Ile Thr Pro Asn Asn Asn Thr Ile Thr Pro Asn Asn Thr Ile Thr Pro Asn Asn Asn Thr Ile Thr Pro Asn Thr Ile Thr Pro Asp Asn Asn Thr Lys Ile Gly Phe Asp Val Gln Ile 1235 1240 1245 Asn Asp Gly Lys Asp Gly Ala Arg Gln Ser Ala Ala Thr Trp Asn Asp 1250 1260 Leu Thr Gly Leu Gly Tyr Gln Asp Thr Ser Val Phe Gly Val Leu Thr 1270 1275 128 Leu Met Lys Thr Asp Thr Thr Ala Pro Val Thr Thr Asp Asn Gly Pro
1285 1290 1295 Glu Asp Trp Val Asn Lys Asp Val Thr Ile Ala Phe Ser Ala Asn Asp 1300 1305 1310 Asn Asp Thr Gly Val Ala Ala Thr Tyr Tyr Ser Ile Asp Asn Gly Val 1315 1320 1325

Val Gln Asn Gly Asn Ser Val Thr Ile Ser Glu Glu Gly Val His Ile 1330 1340 Leu Thr Tyr Trp Ser Val Asp Lys Ala Gly Asn Val Glu Gln Val His 1345 1350 1355 136 1360 Thr Lys Thr Ile Lys Leu Asp Lys Thr Gly Pro Ile Leu Asp Ile Lys 1365 1370 1375 Leu Asp Lys Thr Thr Leu Ser Pro Val Asn His Lys Met Val Pro Ile 1380 1385 1390 Ser Ala Ala Ile Ser Ala Ser Asp Ala Asp Ser Gly Ile His Ser Val 1395 1400 1405 Val Leu Thr Ser Ile Thr Ser Asn Glu Ser Ile Gln Pro Asp Asp Ile 1410 1415 1420 Page 61

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Gln Asn Ala Asn Tyr Asn Lys Pro Ile Thr Gly Thr Thr Asp Ser Phe
                              1430
                                                            1435
Lys Leu Arg Ala Glu Arg Leu Ala Asn Gly Asn Gly Arg Val Tyr Thr
1445 1450 1455
Ile Thr Tyr Thr Ala Thr Asp Lys Ala Gly Asn Val Thr Thr Lys Ser
                  1460
                                               1465
Val Glu Val Ser Val Pro Arg Asp Asn Ser Lys Lys
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gtttacgatt tccgggtggc tgacgccctg gtcgatttgg cggagcggga aggtttgtt
ttggttggcc acacactgct ctggcatcag cagacgccgg actgggtttt tctggacgag
aagggcgaga ccgccacgg ggagctggtg cacactgcat tggagacgca catccgcac
                                                                                                          240
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                                                                                                          360
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                                                                                                          600
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                                                                                                          840
                                                                                                          900
                                                                                                          960
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Asp Ser Phe Lys Val Gly Val Ala Leu Asn Ala Asp Gln Ile Thr Gly
                                         40
Ala Asp Ser Ala Ser Leu Asp Leu Ser Leu Ala His Phe Asp Ser Leu 50 60
Val Ala Glu Asn Ala Met Lys Trp Gly Ser Leu Asn Pro Glu Pro Gly 65 70 75 80
Val Tyr Asp Phe Arg Val Ala Asp Ala Leu Val Asp Leu Ala Glu Arg
Glu Gly Leu Phe Leu Val Gly His Thr Leu Leu Trp His Gln Gln Thr 100 105 110
Pro Asp Trp val Phe Leu Asp Glu Lys Gly Glu Thr Ala Thr Arg Glu
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Leu Val Leu Ala Arg Leu Glu Thr His Ile Arg Thr Val Val Gly Arg
                                                                   140
Tyr Gln Gly Arg Val Gln Gly Trp Asp Val Val Asn Glu Ala Leu Asn
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165 170 175
Asp Tyr Ile Glu Leu Ala Phe Arg Met Ala Lys Glu Ala Asp Pro Asp 180 185 190
Ala Glu Leu Tyr Tyr Asn Asp Tyr Asn Val Ser Lys Pro Gly Lys Arg
Gly Gly Val Val Arg Leu Leu Gly Glu Leu Gln Ala Lys Gly Val Lys 210 220
Val Asp Ala Val Gly Ile Gln Gly His Tyr Ser Leu Gly His Pro Glu
225 230 235 240
Leu Asp Gln Leu Glu Ala Ser Ile Ser Ala Ile Thr Glu Ala Gly Ala
245 250 255
Pro Ile Met Ile Thr Glu Leu Asp Val Ser Val Leu Pro Phe Pro Asp 260 265 270
Ala Glu Gln Met Gly Ala Asp Val Ser Leu Ser Phe Glu Met Gln Asp 285
His Leu Asn Pro Tyr Ala Asp Gly Leu Pro Glu Ala Val Ser Gln Gln 290 295 300
Leu Ala Glu Arg Tyr Ala Ala Ile Phe Glu Val Phe Leu Arg His Gln 305 310 320
Ser His Ile Asp Arg Val Thr Phe Trp Gly Val His Asp Gly Val Ser
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                                                                                                      120
180
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ccaăatcaat tattăgatgc aaăagactca caaatgttaa agcgccatīt taatāgcatī
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                                                                                                     360
                                                                                                      420
                                                                                                      480
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                                                                                                     780
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                                                                                                     960
                                                                                                    1020
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85 90 95 Gln Phe Asn Trp Glu Pro Ala Asp Lys Leu Val Lys Phe Ala Lys Glu 100 105 110 Asn Gly Met Asp Met Arg Gly His Thr Leu Val Trp His Ser Gln Val Pro Asp Trp Phe Phe Lys Asp Ala Asn Gly Asn Ser Met Val Val Trp Gln Asn Gly Lys Gln Val Val Ala Asp Pro Ser Asn Leu Glu Ala Asn 145 150 155 160 Lys Lys Leu Leu Ser Arg Leu Glu Thr His Val Asn Thr Val Val Ser Arg Tyr Lys Asn Asp Ile Lys Phe Trp Asp Val Val Asn Glu Val The Asp Glu Trp Gly Gly His Pro Glu Gly Leu Arg Gln Ser Pro Trp 200 205 Phe Leu Ile Thr Gly Thr Asp Tyr Ile Lys Val Ala Phe Glu Thr Ala 210 _ 215 _ 220 Arg Gln Tyr Ala Ala Pro Asp Ala Lys Leu Tyr Ile Asn Asp Tyr Asn 235 240 Thr Glu Val Thr Pro Lys Arg Thr Tyr Leu Tyr Asn Leu Val Lys Ser Leu Lys Gln Gln Gly Val Pro Ile Asp Gly Val Gly His Gln Ser His 260 265 270 Ile Gln Ile Gly Trp Pro Ser Glu Lys Glu Ile Glu Asp Thr Ile Asn 275 280 285 Met Phe Ala Glu Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val Ser Met Tyr Gly Trp Pro Val Arg Ala Tyr Pro Thr Tyr Asp Ser Ile 305 310 315 320 Pro Ala Gln Lys Phe Ile Asp Gln Ala Asp Arg Tyr Asp Arg Leu Phe 325 Lys Leu Tyr Glu Lys Leu Gly Asp Lys Ile Ser Asn Val Thr Phe Trp 340 345 Gly Ile Ala Asp Asn His Thr Trp Leu Asn Asp Arg Ala Asp Val Tyr 355 360 365 Tyr Asp Ala Asp Gly Asn Val Val Thr Leu Ala Asn Ala Pro Tyr Ala 370 380 Lys Met Glu Ala Arg Ser Gly Lys Asp Ala Pro Phe Val Phe Asp Pro 385 390 400 390 Glu Tyr Asn Val Lys Pro Ala Tyr Trp Ala Île Île Asp His Lys

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60

120 180

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                                                                                                       420
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 gatggtgcca ccggctggga ttgggtcatc acctcgttcc gtctggcgcg tcagtactgt
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                                                                                                       720
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Met Ala Ala Gly Lys Glu Lys Phe Val Gly Asn Val Ile Ala Gly Tyr
Val Pro Gly Asp Tyr Gly Asn Leu Trp Asn Gln Val Thr Pro Glu Asn 50 55 60
Ser Thr Lys Trp Gly Ala Val Glu Ser Thr Arg Asn Val Met Asn Trp 65 70 75 80
Thr Gln Ala Asp Leu Ala Tyr Asn Tyr Ala Lys Ser Lys Gly Phe Lys

85

90

95
Phe Lys Met His Thr Leu Val Trp Gly Ser Gln Glu Pro Ala Trp Val
Lys Asn Leu Asp Ala Thr Ser Gln Arg Val Glu Val Glu Gln Trp Met
115 120 125
Arg Leu Ser Cys Glu Arg Tyr Pro Asp Ser Trp Ala Ile Asp Val Val
Asn Glu Pro Leu His Ala Val Pro Ser Tyr Lys Asn Ala Leu Gly Gly
145 _____ 150 ____ 155 ____ 160
Asp Gly Ala Thr Gly Trp Asp Trp Val Ile Thr Ser Phe Arg Leu Ala
165 170 175
Arg Gln Tyr Cys Pro Arg Ala Lys Leu Leu Leu Asn Glu Tyr Ala Thr
180 185 190
Glu Leu Asp Ala Ser Lys Arg Ala Lys Ile Lys Thr Ile Ala Ser Leu
195 200 205
Leu Lys Ser Arg Gly Leu Ile Asp Gly Val Gly Leu Gln Ala His Phe 210 220
Phe Thr Leu Asp Tyr Met Asn Ala Ser Gln Met Lys Ala Ala Leu Asp 225 230 235
Asp Tyr Ala Thr Leu Gly Val Asp Ile Tyr Ile Ser Glu Leu Asp Leu 255 250
Lys Gly Ser Ala Asn Thr Asp Ala Ser Gln Lys Ala Lys Tyr Glu Glu 260 270
           Pro Val Met Trp Asn His Ala Ser Val Lys Gly Ile Thr Leu 275 285
Trp Gly Tyr Lys Val Gly Glu Thr Trp Ser Ser Gly Thr Gly Leu Leu
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290
                                 295
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325 330 _ 335
Ser Ser Lys Ser Ser Ser Ser Ser Ser Ser Gln Ser Ser Ala Ser Ser 340 345 350
Ser Ala Gly Ser Ala Pro Val Leu Ser Gly Thr Ser Asp Tyr Pro Ser
Gly Phe Ser Lys Cys Ala Asp Leu Gly Gly Thr Cys Ser Val Ser Ser
370 375 380
Gly Thr Gly Trp Ala Ala Phe Gly Arg Lys Gly Lys Trp Val Ala Lys 385 390 395 400
Tyr Val Gly Val Gly Lys Ser Ile Pro Cys Thr Val Ala Ala Phe Gly
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410
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cacttcaaca gtattacggc agagaatgaa atgaagtttg ccagtctgca gccggaggag
                                                                                               180
240
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Phe Asp Glu Ala Asp Arg Leu Ala Ala Phe Ala Arg Lys His Gly Met 65 70 75 80
Ala Met Arg Gly His Thr Leu Val Trp His Asn Gln Ser Thr Gly Trp 85 90 95
Leu Phe Glu Asp Lys Gln Gly Asn Pro Val Asp Lys Ala Thr Leu Leu 100 105
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180 185 190
Lys Ile His Ala Leu Val Lys Ser Leu Leu Glu Gln Gly Val Pro Ile
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Gly Glu Ile Arg Ala Ala Leu Glu Lys Tyr Ala Ser Leu Gly Leu Gln
225 230 235 240
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Glu Arg Tyr Glu Glu Ile Phe Lys Leu Leu Arg Glu Tyr Arg Asp Val
275 280 285
Ile Thr Ser Val Thr Phe Trp Gly Ala Ala Asp Asp Tyr Thr Trp Leu 290 295 300
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PCT/US03/19153 WO 03/106654

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Arg Glu Phe Asn Met Ile Thr Ala Glu Asn Glu Met Lys Ile Asp Ala
65 70 75 80
Thr Glu Pro Asn Gln Asn Gln Phe Asn Phe Thr Asn Ala Asp Arg Ile
Phe Asn Trp Ala Val Gln Asn Gly Lys Gln Val Arg Gly His Thr Leu
100 105 110
Ala Trp His Ser Gln Gln Pro Gly Trp Met Ser Ser Met Ser Gly Thr
115 120 125
Ala Leu Arg Asn Ala Met Ile Asn His Ile Asn Gly Val Met Ala His
130 140
Tyr Lys Gly Arg Ile Tyr Ala Trp Asp Val Val Asn Glu Ala Phe Asn 145 150 160
Glu Asp Gly Ser Arg Arg Asn Ser Asn Leu Gln Gln Thr Gly Asn Asp
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Trp Ile Glu Val Ala Phe Arg Thr Ala Arg Thr Ala Asp Pro Ala Ala
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Lys Leu Cys Tyr Asn Asp Tyr Asn Ile Glu Ala Trp Ser Tyr Ala Lys
Thr Gln Gly Val Tyr Arg Met Val Gln Asp Phe Lys Ser Arg Gly Val 210 215 220
Pro Ile Asp Cys Val Gly Phe Gln Ser His Phe Asn Ser Gly Thr Ser 225 230 235
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Tyr Val Asn Ser Asn Phe Arg Thr Thr Leu Gln Ser Phe Ala Ala Leu
245 250 255
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Arg Cys Asn Gly Ile Thr Val Trp Gly Val Arg Asp Ser Asp Ser Trp 290 295 300 Arg Ser Ser Gln Asn Pro Leu Leu Phe Asn Ser Ser Gly Gly Lys Lys 305 310 320 Ala Ser Tyr Thr Ala Val Leu Asp Ala Leu Asp Ala Ala Pro Thr Val Thr Pro Pro Val Thr Thr Pro Pro Val Thr Thr Pro Pro Val Thr Thr 340 350 Pro Pro Gly Thr Val Ser Ile Asn Ala Gly Gly Ser Ala Ser Gly Ser Phe Thr Ala Asp Gln Tyr Phe Ser Gly Gly Ser Thr Tyr Thr Asn 370 375 380 Thr Ala Thr Ile Asp Met Ser Gln Ile Thr Ser Asn Pro Pro Pro Ala 385 400 Ala Val Phe Asn Ser Glu Arg Tyr Gly Ala Met Thr Tyr Thr Ile Pro Asn Arg Ser Gly Ala Gln Thr Val Thr Leu Tyr Phe Ala Glu Thr Tyr
420 430 Leu Thr Ala Ala Gly Gln Arg Ser Phe Asn Val Ser Ile Asn Gly Ala
435
440
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725
730
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                                                                     845
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885 890 895
Ile Ala Cys Ala Arg Ala Asn Val Phe Arg Gly Val Ala Leu Tyr Ala
900 905 910
                                              905
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915 920 925
Phe Ala Thr His Gly Ile Asn Asp Ser Val Leu Asn Ile Ser Gln Gly 930 940
Arg Thr Leu Arg Asp Arg Phe Val Ser Asn Asn Ser Cys Thr Ala Gln
945 950 955 960
Asn Pro Pro Glu Pro Ser Ser Gly Ser Gly Thr His Ile Cys Thr Ser
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Val Lys Val Val Asp Thr Thr Ser Ala Glu Ile Lys Leu Glu Met Asn 50 _ _ _ 60
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130 140
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Glu Trp Phe Phe Lys Asp Glu Asn Gly Asn Leu Leu Ser Lys Glu Ala
Met Thr Glu Arg Leu Arg Glu Tyr Ile His Thr Val Val Gly His Phe
180

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Ile Asp Gly Ile Gly Met Gln Cys His Ile Ser Leu Ala Thr Asp Ile
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Trp Gly Ser Ile Glu Gly Asn Arg Asn Gln Met Asn Trp Gly Asn Ala 65 70 _ 75 80
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115 120 125
Gly Gln Arg Tyr Ser Ala Lys Thr Ala Phe Val Asp Val Val Asn Glu
130 135 140
Pro Leu His Ala Lys Pro Ser Tyr Arg Asn Ala Île Gly Gly Asp Gly 145 150 155 160
Ser Thr Gly Trp Asp Trp Val Ile Trp Ser Phe Gln Gln Ala Arg Ala 165 170 175
Ala Phe Pro Asn Ala Lys Leu His Leu Asn Asp Tyr Gly Ile Ile Gly
                                    185
Asp Pro Ser Ala Ala Asp Lys Tyr Val Asn Ile Ile Asn Ile Leu Lys
195 200 205
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325 330 335
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1080

1089

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Glu Gln Gly Arg Tyr Tyr Asp Val Asn Gln Île Île His Arg His 340 350 Ala Asp Gln Leu Phe Ser Val Ser Val Trp Gly Leu Ser Asp Asp Gln 355 360 365 Ser Trp Arg Asn Lys Glu Gly Ala Pro Leu Leu Phe Asp Asp Asn Leu 370 380 Glu Lys Lys Pro Ala Tyr Ile Gly Tyr Ile Gly Asp Ser Ala Asn Leu 385 390 395 400 Pro Glu Pro Leu Lys Ser Met Asn Ala Phe Lys Asp Asp Ala Val Gly
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520
525 Val Glu Met Asn Val Ile Ala Thr Asp Ser Ala Thr Thr Glu Thr Ser 530 540 Ala Trp Ser Thr Asn Asp Thr Gly Ala Val Thr Leu Ala Glu Pro Leu 545 550 550 560 Ser Tyr Thr Glu Ala Val Lys Val Pro Ala Asp Ala Gln Ala Pro Val 565 575 Val Asp Ala Asp Pro Ser Asp Ser Val Trp Ala Glu Ala Asn Glu Val 580 585 Pro Val Gly Lys Val Thr Ala Ala Thr Pro Ser Pro Glu Ala Thr Ala
595 600 605 Thr Ala Lys Thr Leu Trp Ser Asp Gly Lys Leu Tyr Val Leu Met Glu 610 620 Val Thr Asp Ala Asp Ile Asp Leu Thr Asn Ser Asn Pro Trp Glu Lys 625 630 640 Asp Ser Val Glu Val Tyr Ile Asp Arg Gly Asn Thr Lys Ser Gly Gln
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Tyr Thr Asn Asp Ile Gln Gln Ile Arg Val Ser Ala Asp Gly Ala Glu
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665
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Gln Ile Asn Asp Ala Lys Asn Gly Ala Arg Ile Gly Ile Arg Asn Trp
Ala Asp Pro Thr Gly Ala Gly Tyr Gln Thr Ala Ser His Trp Gly Val
740 745 750
Leu Arg Leu Leu Ala Asp Pro Ser Glu Thr Glu Thr Pro Gly Gly Glu 755 760 765
Asp Pro Glu Thr Pro Gly Asp Glu Glu Thr Pro Gly Glu Asp Thr Glu 770 780
Lys Pro Gly Asp Glu Glu Thr Pro Gly Glu Asp Thr Glu Lys Pro Gly 785 790 795 800
Asp Glu Lys Pro Arg Pro Ser Asp Asp Ala Asp Asn Asp Asp Lys Met
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Gln Glu Ser Leu Leu Thr His His Phe Asn Ser Ile Thr Ala Glu Asn
35 40 45
Glu Met Lys Phe Ala Ser Val His Pro Glu Glu Glu Leu Tyr Thr Phe 50 60 60
Glu Glu Ala Asp Gln Ile Val Asp Phe Ala Arg Lys His Gly Met Ala 65 70 80
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Val Arg Gly His Thr Leu Val Trp His Asn Gln Thr Thr Asp Trp Leu
Phe Arg Asp Lys Gln Asn Gln Leu Val Ser Lys Ala Val Leu Tyr Glu
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Arg Ile Arg Ser His Ile Gln Thr Val Val Gly Arg Tyr Lys Gly Asp
Ile Tyr Ala Trp Asp Val Val Asn Glu Val Ile Ala Asp Asp Gly Asp
130 135 140
Gln Leu Leu Arg Thr Ser Ser Trp Thr Glu Ile Ala Gly Asp Glu Phe
145 150 155 160
Ile Ala Lys Ala Phe Glu Tyr Ala His Ala Ala Asp Pro Asn Ala Leu
                                            170
Leu Phe Tyr Asn Asp Tyr Asn Glu Ser His Pro Ser Lys Arg Asp Lys
180 185 190
                                       185
              180
Ile Tyr Thr Leu Val Lys Ser Leu Leu Asp Arg Gly Val Pro Ile His
195 200 205
Gly Ile Gly Leu Gln Ala His Trp Asn Leu Phe Asn Pro Ser Leu Asp
                             215
                                                      220
Asp Ile Arg Ala Ala Ile Glu Lys Tyr Ala Ser Leu Gly Leu Gln Leu
225 230 235 240
Gln Leu Thr Glu Leu Asp Val Ser Val Phe Arg Phe Glu Asp Lys Arg
245 250 255
Ala Asp Leu Thr Glu Pro Glu Pro Gly Met Leu Glu Gln Gln Ala Glu
260 265 270
Phe Tyr Glu Ala Val Phe Lys Leu Leu Lys Glu Tyr Ser Asp Val Ile 275 280 285
Ser Ala Val Thr Phe Trp Gly Ala Ala Asp Asp His Thr Trp Leu Ser
290 295 300
Asp Phe Pro Val Arg Gly Arg Lys Asn Trp Pro Leu Leu Phe Asp Glu
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His Leu Arg Arg
<210> 93
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caaaaagacg gtacgacaat tgatcgtgaa acactcttgg agagaatgaa aaaacatatt
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gattatacat ggcttgatga ttttccggtg acaggtcgaa aaaattggcc ctttgtattt
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<212> PRT
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<213> Unknown

<400> 94

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 Glu Asn Glu Met Lys Phe Glu Ala Leu Gln Pro Lys Pro Asp Gln Phe
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55
60
 Thr Phe Asp Thr Ala Asp Lys Met Val Ala Phe Ala Gln Ala His Asp 65 70 75 80
Met Lys Met Arg Gly His Thr Leu Ile Trp His Asn Gln Thr Pro Asp 85 90 95
Trp Met Phe Leu Gln Lys Asp Gly Thr Thr Ile Asp Arg Glu Thr Leu 100 105 110
Leu Glu Arg Met Lys Lys His Ile Lys Thr Val Val Glu Arg Tyr Lys
115
120
125
Gly Lys Ile Tyr Cys Trp Asp Val Val Asn Glu Ala Val Ala Asp Glu
130 140
Gly Glu Ala Ile Leu Arg Pro Ser Lys Trp Thr Asp Ile Ile Gly Asp 155 160
Ser Phe Ile Glu Tyr Ala Phe Lys Tyr Ala His Glu Ala Asp Pro Asp
165 170 175
Ala Leu Leu Phe Tyr Asn Asp Tyr Asn Ala Cys His Pro His Lys Arg
Asp Lys Ile Tyr Gln Leu Val Lys Gly Leu Ile Asp Lys Gly Val Pro
195 200 205
Ile His Gly Ile Gly Leu Gln Ala His Trp Asn Ile Val Asp Pro Ser 210 220
Tyr Asp Asp Ile Lys Arg Ala Ile Glu Thr Tyr Ala Ser Leu Gly Leu 225 230 240
Ser Ile His Phe Thr Glu Met Asp Val Ser Val Phe Glu Tyr His Asp 245 250 255
Arg Arg Thr Asp Leu Leu Glu Pro Thr Lys Asp Met Val Ser Arg Gln 260 265 270
Ala Glu Arg Tyr Gln Ala Phe Phe Glu Ile Phe Arg Ser Tyr Ala Asp
275 280 285
Val Ile Asp Ser Val Thr Phe Trp Gly Met Ala Asp Asp Tyr Thr Trp 290 295 300
Leu Asp Asp Phe Pro Val Thr Gly Arg Lys Asn Trp Pro Phe Val Phe 305
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720
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960

1020

1080 1140

1143

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Gly Thr Ala Met Asn Leu Arg Gln Ile His Gly Asp Asp Pro Gln Ser 50 55 60
Glu Asn Ile Ile Lys Lys Gln Phe Asn Ser Ile Val Ala Glu Asn Cys
65 70 75 80
Met Lys Ser Met Tyr Leu Gln Pro Glu Glu Gly Lys Phe Phe Asp 85 90 95
Asp Ala Asp Lys Phe Val Asp Phe Gly Leu Gln Asn Asn Met Phe Ile
100 105 110
Ile Gly His Cys Leu Ile Trp His Ser Gln Ala Pro Lys Trp Phe Phe 115 120 125
Thr Asp Glu Asn Gly Lys Thr Val Ser Pro Glu Val Leu Lys Gln Arg
130 135 140
Met Lys Ala His Ile Thr Ala Val Val Ser Arg Tyr Lys Gly Lys Ile
145 150 155 160
Lys Gly Trp Asp Val Val Asn Glu Ala Ile Met Glu Asp Gly Ser Tyr
Arg Lys Ser Lys Phe Tyr Glu Ile Leu Gly Glu Glu Phe Ile Pro Leu
180 185 190
Ala Phe Gln Tyr Ala His Glu Ala Asp Pro Asp Ala Glu Leu Tyr Tyr
195 200 205
Asn Asp Tyr Asn Glu Trp Tyr Pro Gly Lys Arg Ala Thr Val Thr Lys 210 220
Ile Ile Arg Asp Phe Lys Ser Arg Gly Ile Arg Ile Asp Ala Ile Gly 225 230 230 240
Met Gln Ala His Phe Gly Met Asp Ser Pro Thr Leu Glu Glu Tyr Glu
245 250 255
Gin Thr Ile Gin Gly Tyr Ile Lys Glu Gly Val Lys Val Asn Ile Thr
260 265 _ . 270 _
Glu Leu Asp Leu Ser Pro Leu Pro Ser Pro Trp Gly Thr Ser Ala Asn 275 280 285
Val Ala Asp Thr Gln Gln Tyr Gln Glu Lys Met Asn Pro Tyr Thr Lys
290 295 300
Gly Leu Pro Ala Asp Val Glu Lys Ala Trp Glu Asn Arg Tyr Leu Asp 305 310 315 320
Phe Phe Lys Leu Phe Leu Lys Tyr His Gln His Ile Glu Arg Val Thr
325 330 335
Phe Trp Gly Val Ser Asp Ile Asp Ser Trp Lys Asn Asp Phe Pro Val
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35 40 45 Arg Phe Ala Gln Asp Arg Tyr Asn Pro Phe Arg Ile Gly Phe Leu Pro 50 60 Phe Pro Pro Arg Val Ala Pro Ala Ala Gln Pro Trp Ala Asp Gly Ser 65 70 75 80 Glu Arg Arg Trp Arg Ser Arg Lys Pro Ala Lys Lys Gln Leu Ala Phe 85 90 95 Leu Ala Ile Thr Ser Leu Leu Ser Gly Leu Leu Trp Gly Ala Glu Val 100 105 110 Gln Pro Ala Leu Lys Asp Val Phe Arg Gln Asp Phe Leu Leu Gly Ala 120 125 Ala Leu Asn Ala Glu Gln Val Leu Asp Thr Asn Arg Val Glu Ser Val 130 140 Leu Ile Glu Lys His Phe Asn Thr Ile Thr Pro Glu Asn Val Leu Lys 155 Trp Glu Arg Val His Pro Gln Pro Asn Gln Tyr Ser Phe Glu Asp Ala 165 170 175 Asp Arg Tyr Val Glu Phe Gly Arg Lys His Gly Met Val Ile Ile Gly 180 185 190 His Thr Leu Val Trp His Ser Gln Thr Pro Gly Trp Val Phe Arg Asp 195 200 205

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Ala Asp Gly Lys Thr Leu Thr Arg Glu Ala Leu Leu Glu Arg Met Arg 210 215 220

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Asp His Ile His Thr Val Val Gly Arg Tyr Lys Gly Lys Ile Arg Gly 225 230 240
Trp Asp Val Val Asn Glu Ala Leu Arg Asp Asp Gly Ala Trp Arg Asn 245 250 255
Ser Gln Trp Arg Arg Ile Ile Gly Asp Asp Tyr Ile Leu Lys Ala Phe
Gln Tyr Ala His Glu Ala Asp Pro Asp Ala Glu Leu Tyr Tyr Asn Asp 275 _ 280 _ 285
 Tyr Ser Leu Glu Lys Pro Ala Lys Arg Asn Gly Ala Val Asp Leu Val
290 295 300
Lys Gln Leu Gln Ala Gly Gly Ala Lys Leu Ala Gly Val Gly Leu Gln 305 310 320
Gly His Tyr Asn Leu Asp Trp Pro Glu Thr Ala Glu Ile Glu Asn Thr
325 330 335
Ile Ala Ala Phe Ala Glu Leu Gly Leu Lys Val Met Ile Thr Glu Leu 340 345 350
Asp Val Asn Ala Leu Pro Thr Pro Gly Gln Ser Gly Glu Ala Asp Val 355 360 365
Gly Met Thr Phe Gly Gly Asn Phe Gly Gly Asp Lys Trp Asn Pro Phe 370 380
Thr Asn Gly Leu Pro Ala Ala Val Glu Gln Arg Leu Ala Asp Arg Tyr 385 ____ 390 395 400
Ala Glu Ile Phe Arg Ile Phe Thr Lys His Ser Arg Arg Ile Ser Arg
405 410 415
Val Thr Phe Trp Gly Val Thr Asp Arg Thr Ser Trp Leu Asn Asn Phe
420
425
430
Pro Ile Arg Gly Arg Thr Asn Tyr Pro Leu Leu Phe Asp Arg Ala Gly
435 440 445
Glu Pro Lys Pro Ala Phe Arg Ser Val Val Ala Val Arg Gln Pro Arg
450 455 460
Gln Pro Val Glu
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ggagccgcgg tggacctagc ggccctgtac gaccccctcg agcccgagta cgcccaactc
ctcgcccgcg agttcaacct ggtggtggcc gagaacgcca tgaagtgggc ctccctgagc
                                                                                                              120
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                                                                                                              240
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                                                                                                              420
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                                                                                                              660
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Leu Tyr Asp Pro Leu Glu Pro Glu Tyr Ala Gln Leu Leu Ala Arg Glu
50 55 60
Phe Asn Leu Val Val Ala Glu Asn Ala Met Lys Trp Ala Ser Leu Ser
65 70 75 ____ 80
Asn Ala Arg Gly Gln Tyr Ser Phe Thr Gly Ala Asp Ala Leu Val Arg
85 90 95
Phe Ala Arg Gln His Gly Gln Arg Leu Arg Gly His Thr Leu Ile Trp
              100
                                      105
His Glu Gln Leu Pro Ala Trp Val Arg Ser Gly Thr Phe Ser Arg Glu
                                 120
         115
Ala Met Leu Ala Val Met Gln Glu His Ile Gln Ala Val Ala Gly His
                            135
                                                    140
Phe Arg Gly Gln Val Ala Tyr Trp Asp Val Val Asn Glu Ala Val Ser
145 150 155 160
Asp Arg Gly Gly Leu Arg Glu Thr Pro Phe Leu Arg Ala Val Gly Pro
165 170 175
Asp Tyr Leu Glu His Ala Phe Arg Phe Ala Arg Ala Ala Asp Pro Gln
180 185 _ _ _ 190 _
Ala Lys Leu Phe Tyr Asn Asp Tyr Gly Ala Asp Gly Met Gly Ala Lys
195 200 205
Ser Asp Glu Ile Tyr Ala Leu Leu Lys Ala Leu Lys Ala Lys Gly Val
Pro Val Asp Gly Val Gly Phe Gln Ala His Leu Asp Ser Thr Phe Ser 225 235 240
Val Gln Gln Ala Arg Met Arg Glu Asn Leu Glu Thr Leu Arg Arg Pro
255 255
Gly Pro Arg Gly Ala His His Arg Ala Gly Arg Ala Ala Lys Arg Gly
260 265 270
Gly Leu Ala Gly Gly Thr Ala Gly Gly Ala Gly Pro Asp Leu Arg Arg 275 280 285
Gly Ala Gly Asp Leu Pro Arg Gly Pro Arg Leu Gln Arg Arg Asp Ala 290 295 300
Val Gly Leu His Arg Arg Pro Leu Leu Ala Ser Arg Arg Arg Thr Pro
305 310 315 320
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Thr Arg Thr Ala Leu
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atcggtgtgg ctttgaaccg gagtcaatat ctggaacaaa acgaacaggc ggataaagag ataaaggcac agttcagctc tattgtagct gagaactgca tgaaaagcga aaatctggaa cctaaagagg gaaaattctt ctttgacgat gccgatcgtt ttgtcgcttt tggagaaaaa
                                                                                   180
                                                                                   240
                                                                                   300
aatggaatgt acatcattgg acatacctta atttggcatt ctcaagtgcc aaaatggttt
                                                                                   360
ttcatagata atgaaggcaa agttgtttcc cgggaagttt tgattgaacg aatgaaaaac
                                                                                   420
tacatccata cagttgtcgg tcattataaa ggtcgagtta aaggttggga tgttgtcaat
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gaggccattc tagatgatgg ctcatttaga caaagtaatt tctttaaaat actaggagcc

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480

540

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                                                                                  960
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Gln Tyr Leu Glu Gln Asn Glu Gln Ala Asp Lys Glu Ile Lys Ala Gln
50 55 60
Phe Ser Ser Ile Val Ala Glu Asn Cys Met Lys Ser Glu Asn Leu Glu 65 75 80
Pro Lys Glu Gly Lys Phe Phe Phe Asp Asp Ala Asp Arg Phe Val Ala 85 90 95
Phe Gly Glu Lys Asn Gly Met Tyr Ile Ile Gly His Thr Leu Ile Trp
His Ser Gln Val Pro Lys Trp Phe Phe Ile Asp Asn Glu Gly Lys Val
115 120 125
Val Ser Arg Glu Val Leu Ile Glu Arg Met Lys Asn Tyr Ile His Thr
130 140
Val Val Gly His Tyr Lys Gly Arg Val Lys Gly Trp Asp Val Val Asn
150 155 160
Glu Ala Ile Leu Asp Asp Gly Ser Phe Arg Gln Ser Asn Phe Phe Lys
165 170 175
Ile Leu Gly Ala Asp Phe Ile Lys Leu Ala Phe Gln Phe Ala His Glu
180 185 190
Ala Asp Pro Asn Ala Glu Leu Tyr Tyr Asn Asp Tyr Ser Met Ser Asn
195 200 205
Pro Thr Lys Arg Asp Gly Val Val Arg Met Val Lys Ser Leu Gln Gln 210 220
Gln Gly Val Arg Ile Asp Ala Ile Gly Met Gln Gly His Val Gly Met
225 230 235 240
Asp Tyr Pro Lys Leu Asp Glu Phe Glu Asn Ser Ile Lys Ala Phe Ser 255
Pro Thr Pro Lys Gly Lys Gln Gly Ala Asn Ile Ser Asp Val Ala Ala
275 280 285
Tyr Glu Glu Lys Ile Asn Pro Tyr Lys Asn Gly Leu Pro Ala Glu Val
290 295 300
Glu Lys Ala Trp Glu Asp Arg Tyr Leu Asp Phe Phe Lys Leu Phe Leu 305 310 315
Lys Tyr Gln His Gln Ile Ser Arg Val Thr Leu Trp Gly Leu Ser Asp
325 330
Gln Asp Ser Trp Lys Asn Asp Phe Pro Val Arg Gly Arg Thr Asp Tyr 340 345 350
         Leu Phe Asp Arg Gln Tyr Lys Pro Lys Pro Val Val Gln Lys 355
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                                                                                                         360
                                                                                                         420
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gacggttcca tcgtcggtgt ccagtccggc ctctgcctcg acgccgctgc cggcggcacc
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Leu Val Ala Pro Leu Thr Ser His Ala Ala Glu Ser Thr Leu Gly Ala
                                         40
Ala Ala Lys Gln Ser Gly Arg Tyr Phe Gly Thr Ala Ile Ala Ser Gly 50 60
Arg Leu Asn Asp Ser Thr Tyr Thr Thr Ile Ala Asn Arg Glu Phe Asn 75 75 80
Ser Val Thr Ala Glu Asn Glu Met Lys Ile Asp Ala Thr Glu Pro Gln
85 90 95
Gln Gly Arg Phe Asp Phe Thr Ala Gly Asp Arg Val Tyr Asn Trp Ala
100 105 110
Val Gln Asn Gly Lys Gln Val Arg Gly His Thr Leu Ala Trp His Ser
115 120 125
Gln Gln Pro Ala Trp Met Gln Asn Leu Ser Gly Ser Ala Leu Arg Thr
                                                                 140
Ala Met Thr Asn His Ile Asn Gly Val Met Ala His Tyr Lys Gly Lys
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Ile Gly Gln Trp Asp Val Val Asn Glu Ala Phe Ala Asp Gly Ser Ser
165 170 175
 Gly Ala Arg Arg Asp Ser Asn Leu Gln Arg Ser Gly Asn Asp Trp Ile
180 185 190
 Glu Val Ala Phe Arg Thr Ala Arg Ala Ala Asp Pro Ala Ala Lys Leu
195 200 205
 Cys Tyr Asn Asp Tyr Asn Val Glu Asn Trp Thr Trp Ala Lys Thr Gln
210 220
 Ala Met Tyr Ala Met Val Lys Asp Phe Lys Gln Arg Gly Val Pro Ile
225 230 235 240
Asp Cys Val Gly Phe Gln Ser His Phe Asn Asn Asp Ser Pro Tyr Asn 245 250 255
Ser Asn Phe Arg Thr Thr Leu Gln Ser Phe Ala Ala Leu Gly Val Asp 260 270
Val Ala Ile Thr Glu Leu Asp Ile Gln Gly Ala Ser Gly Thr Thr Tyr
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280
285
Ala Asn Val Thr Asn Asp Cys Leu Ala Val Pro Arg Cys Leu Gly Ile
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Thr Val Trp Gly Val Arg Asp Thr Asp Ser Trp Arg Ala Glu His Thr 305 310 315 320
Pro Leu Leu Phe Asn Gly Asp Gly Ser Lys Lys Pro Ala Tyr Ser Ser 325 330 335
Val Leu Asn Ala Leu Asn Ser Val Ser Pro Asn Pro Asn Pro Thr Pro
Thr Pro Ser Pro Gly Ala Gly Pro Ile Lys Gly Val Ala Ser Gly Arg
Cys Val Asp Val Pro Gly Ala Gly Thr Ala Asp Gly Thr Gln Val Gln 370 380
Leu Trp Asp Cys Asn Asn Arg Thr Asn Gln Gln Trp Thr Leu Thr Ala 385 390 395 400
Ala Gly Glu Leu Arg Val Tyr Gly Asp Lys Cys Leu Asp Ala Ala Gly
405 415
Thr Gly Asn Gly Ala Lys Val Gln Ile Tyr Ser Cys Trp Gly Gly Asp
420
430
Asn Gln Lys Trp Arg Leu Asn Ser Asp Gly Ser Ile Val Gly Val Gln
435 440 445
Ser Gly Leu Cys Leu Asp Ala Ala Ala Gly Gly Thr Ala Asn Gly Thr
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<211> 2793
<212> DNA
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                                                                                                 120
gccgtcagct tggcgcaact gcaagcatcg aaaaaccatg aacgagattt aatcgcccag cactttaaca gtctgaccgc tgaaaacctg atgaaatggg aaaaaatcca accgactgaa ggcaactttg atttacagc ggccgacaag ctcgtcgctt ttgctgaaca acatcggatg tggctggtcg gccatacgat ccaaggatg tacaacaccac cggatgggat attcaggg
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                                                                                                 240
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                                                                                                 360
ccagatggca aaccggccag caagcaagtg ttactcggca gattaaaaaa gcatatccaa
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                                                                                                 600
                                                                                                 660
caacaacaag tgcccattca tgccattggc gaacaagcgc attatggtct cgattcgccg
                                                                                                 720
aaattgcagg aagttgaaga ctcgatcaac gcctttgcag ccaccggcct cgacgtgatg ctgaccgagt tggaaatttc ggtgctaccg tttccgcctg gcatgacacc aggcgccgat atcagtcagc atcaggaact gcaacaacag ctgaatcctt accgcgaagg cttaccaaaa
                                                                                                 780
                                                                                                 840
                                                                                                 900
accgicgaac aggcciggca acaacgttai ciggatcigi titcgcigit attgcgccag
                                                                                                 960
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1320

2760 2793

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 Gln His Arg Met Trp Leu Val Gly His Thr Ile Leu Trp His Glu Gln
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Asp Asp Tyr Ile Ala Thr Thr Phe Ala Leu Val His Gln Val Asp Pro 180

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545 550 560 Asp Arg Thr Trp Asn Leu Pro Glu Ser Ser Asn Asn Leu Pro Asp Ile 565 570 575 Leu Asp Glu Thr Leu Trp Asn Leu Gln Trp Leu Ser Thr Met Gln Asp 580 585 590 Pro Ser Asp Gly Gly Val Tyr His Lys Leu Thr Glu Leu Asn Phe Ser 595 600 605 Ala Thr Gln Met Pro Ser Glu Val Thr Ala Pro Arg Tyr Val Val Gln 610 620 Lys Thr Thr Ala Ala Ala Leu Asn Phe Ala Ala Val Leu Ala Lys Ala 625 630 640 Ser Arg Ile Phe Thr Glu Phe Glu Thr Gln Leu Pro Gly Leu Ser Gln 645 655 Gln Tyr Arg Gln Gln Ala Leu Ala Ala Trp Gln Trp Ala Gln Lys Asn
660 665 670 Pro Gln Gln Ile Tyr Gln Gln Pro Ala Asp Val His Thr Gly Ala Tyr
675 680 685 Gly Asp Lys Gln Leu Ala Asp Glu Trp Ala Trp Ala Gly Ala Glu Leu
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Thr Gly Phe Gly Ala Gln Ser Pro Gln His Ile His His Arg Pro Ser
835 840 845
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PCT/US03/19153 WO 03/106654

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 Lys Asp Ser Phe Thr Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Leu 50 60
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180 185 190
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Pro Glu Leu Lys Phe Met Asp Gln Ala Ala Arg Tyr Asp Arg Leu Phe 325 330 335
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Tyr Asp Glu Asn Gly Asn Val Val Leu Asp Arg Glu Thr Pro Arg Val
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Lys Glu Gly Glu Phe Asp Phe Ala Asp Gly Asp Arg Leu Leu Asp Ile
Thr Gln Gln Cys Gly Ala Thr Ala Ile Gly His Thr Leu Leu Trp His
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960

1020

1080

1140 1155

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Phe Phe Leu Asp Lys Glu Gly Asn Lys Met Val Asp Glu Thr Asp Pro
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Lys Gln Arg Glu Lys Asn Lys Arg Leu Leu Leu Lys Arg Leu Glu Thr
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His Ile Lys Thr Ile Val Lys Arg Tyr Lys Asn Asp Ile Ser Ser Trp
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Lys Glu Leu Val Lys Glu Gly Val Pro Val Asp Gly Val Gly His Gln
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Asp Glu Ile Pro Ala Ser Glu Phe Glu Arg Gln Ala Val Arg Tyr Asp 305 310 _ 315 320
Gln Leu Phe Asp Leu Tyr Glu Arg Leu Gly Asp Lys Ile Ser Ser Val
Thr Phe Trp Gly Val Ala Asp Asn His Thr Trp Leu Asn Asp Arg Ala 340 345 350
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 Ser Ile Met Leu Ala Ala Cys Ser Asn Ala Gln Glu Asn Val Pro Pro 50 55 60 _ _ _
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 Pro Leu Lys Asp Ala Phe Lys Gly Lys Phe Leu Ile Gly Thr Ala Val
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 Ile Lys Thr Val Val Gly Arg Tyr Lys Gly Arg Ile Lys Gln Trp Asp
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Lys Leu Ile Asp Gln Lys Val Pro Ile His Gly Val Gly Ile Gln Ala
275 280 285
His Trp Arg Met Thr Pro Pro Leu Ala Glu Thr Glu Glu Ala Ile Lys
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Leu Pro Ala Glu Val Ala Gln Gln His Ala Glu Arg Tyr Arg Gln Ala
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Phe Glu Leu Phe Leu Arg His Lys Asp Val Ile Gly Arg Val Thr Leu 370 375 380
Trp Gly Thr His Asp Gly Glu Ser Trp Leu Asn Gly Phe Pro Val Arg 385 390 395 400
Gly Arg Thr Asp Tyr Pro Leu Leu Phe Asp Arg Arg Tyr Gln Pro Lys 405 410 415
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180

180

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615

610

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Tyr Thr Ser Ser Ser Gln Arg Tyr Asp Thr Leu Pro Gln Asp Ile Met 770 780
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1740

1800 1860 1905

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Phe Val Pro Ala Lys Arg Glu Gly Ile Ala Arg Met Val Lys Lys Leu 465 470 480
Lys Asp Gln Gly Ile Arg Ile Asp Gly Val Gly Phe Gln Cys His Ile
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Gly Leu Asp Tyr Pro Gly Leu Asp Glu Tyr Glu Lys Thr Ile Gln Leu
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Val Leu Pro Met Pro Asp Trp Arg Val Gly Ala Glu Ile Ser Ala Ser
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Phe Leu Lys Tyr His Glu Val Ile Pro Arg Val Thr Val Trp Gly Val
580 585 590
Asn Asp Gly Asn Ser Trp Lys Asn Gly Phe Pro Val Arg Gly Arg Thr 595 600 605
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Val Gln Met Leu Lys Arg His Phe Asn Ser Ile Val Ala Glu Asn Val
65 70 75 80
Met Lys Pro Ile Asn Ile Gln Pro Glu Glu Gly Lys Phe Asn Phe Ala
85 90 95
Glu Ala Asp Gln Ile Val Arg Phe Ala Lys Lys His His Met Asp Ile
Arg Phe His Thr Leu Val Trp His Ser Gln Val Pro Gln Trp Phe Phe 115 120 125
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Leu Asp Lys Glu Gly Lys Pro Met Val Asn Glu Thr Asp Pro Ala Lys
130 135 140
Arg Glu Gln Asn Lys Gln Leu Leu Leu Lys Arg Leu Glu Ile His Ile
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Lys Thr Ile Val Glu Arg Tyr Lys Asp Asp Ile Lys Tyr Trp Asp Val
Val Asn Glu Val Val Gly Asp Asp Gly Lys Leu Arg Asn Ser Pro Trp
Tyr Gln Ile Ala Gly Ile Asp Tyr Ile Lys Val Ala Phe Gln Thr Ala
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Arg Thr Tyr Gly Gly Asn Lys Ile Lys Leu Tyr Ile Asn Asp Tyr Asn 210 215 220
Thr Glu Val Glu Pro Lys Arg Ser Ala Leu Tyr Asn Leu Val Lys Gln 235 240
Leu Lys Glu Glu Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser His 245 250 255
Ile Gln Ile Gly Trp Pro Ser Glu Glu Glu Ile Glu Lys Thr Ile Asn
260 265 270
Met Phe Ala Asp Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp Val
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Ser Met Tyr Gly Trp Pro Pro Arg Ala Tyr Pro Ser Tyr Asp Ala Ile
290 295 300
Pro Glu Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu Phe 305 310 315
Lys Leu Tyr Glu Lys Leu Gly Asp Lys Ile Ser Asn Val Thr Phe Trp 325 330 335
Gly Ile Ala Asp Asn His Thr Trp Leu Asp Ser Arg Ala Asp Val Tyr
Tyr Asp Ala Asn Gly Asn Val Val Val Asp Pro Asn Ala Pro Tyr Ala 355 360 365
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 Pro Gln Arg Gly Val Tyr Ser Phe Glu Gly Ala Asp Ala Ile Val Arg
 Phe Ala Glu Thr His Gly Met Lys Val Arg Gly His Thr Leu Val Trp 65 70 75 80
 His Gln Gln Leu Pro Ala Trp Ile Thr Ser Gly Ser Tyr Ala Trp Glu
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 Glu Trp Lys Asn Ile Leu Arg Glu His Val Met Ser Val Val Gly Arg
 Tyr Lys Gly Gln Ile Tyr Ala Trp Asp Val Val Asn Glu Ala Ile Leu
115 120 125
 Asp Asn Gly Ser Leu Arg Asp Asn Val Trp Phe Arg Asn Val Gly Pro
 Glu Tyr Ile Glu Ser Ala Phe Arg Trp Ala His Glu Ala Asp Pro Asn
145 150 150 160
 Ala Leu Leu Phe Tyr Asn Asp Tyr Glu Ala Glu Asp Leu Asn Asp Lys
165 170 175
 Ser His Ala Val Tyr Asn Leu Val Lys Ser Leu Leu Glu Lys Gly Val
 Pro Ile His Gly Val Gly Leu Gln Met His Ile Asn Val Glu Asn Pro 195 200 205
 Pro Lys Pro Glu Asp Val Ala Ala Asn Ile Lys Arg Leu Asn Asp Leu 210 220 220
 Gly Leu Ile Val His Ile Thr Glu Met Asp Val Arg Ile Arg Thr Pro
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100 105 110 Glu Ala Asn Thr Val Tyr Ala Ala Asn Ala Val Leu Lys Asp Met Tyr
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125 Ala Asn Tyr Phe Arg Val Gly Ser Val Leu Asn Ser Gly Thr Val Asn 130 140 Asn Ser Ser Ile Lys Ala Leu Ile Leu Arg Glu Phe Asn Ser Ile Thr 145 150 155 160 Cys Glu Asn Glu Met Lys Pro Asp Ala Thr Leu Val Gln Ser Gly Ser 165 170 175 Thr Asn Thr Asn Ile Arg Val Ser Leu Asn Arg Ala Ala Ser Ile Leu 180 185 190 Asn Phe Cys Ala Gln Asn Asn Ile Ala Val Arg Gly His Thr Leu Val Trp His Ser Gln Thr Pro Gln Trp Phe Phe Lys Asp Asn Phe Gln Asp 210 220 Asn Gly Asn Trp Val Ser Gln Ser Val Met Asp Gln Arg Leu Glu Ser 225 230 235 240 Tyr Ile Lys Asn Met Phe Ala Glu Ile Gln Arg Gln Tyr Pro Ser Leu 245 250 255 Asn Leu Tyr Ala Tyr Asp Val Val Asn Glu Ala Val Ser Asp Asp Ala 260 265 270 Asn Arg Thr Arg Tyr Tyr Gly Gly Ala Arg Glu Pro Gly Tyr Gly Asn 275 280 285 Gly Arg Ser Pro Trp Val Gln Île Tyr Gly Asp Asn Lys Phe Île Glu 290 295 300 Lys Ala Phe Thr Tyr Ala Arg Lys Tyr Ala Pro Ala Asn Cys Lys Leu 305 310 _ 315 Tyr Tyr Asn Asp Tyr Asn Glu Tyr Trp Asp His Lys Arg Asp Cys ile
325 330 335 Ala Ser Ile Cys Ala Asn Leu Tyr Asn Lys Gly Leu Leu Asp Gly Val Gly Met Gln Ser His Ile Asn Ala Asp Met Asn Gly Phe Ser Gly Ile 355 360 365 Gln Asn Tyr Lys Ala Ala Leu Gln Lys Tyr Ile Asn Ile Gly Cys Asp 370 380 Val Gln Ile Thr Glu Leu Asp Ile Ser Thr Glu Asn Gly Lys Phe Ser 395 390 Leu Gln Gln Ala Asp Lys Tyr Lys Ala Val Phe Gln Ala Ala Val 405 410 415 Asp Ile Asn Arg Thr Ser Ser Lys Gly Lys Val Thr Ala Val Cys Val 420 425 430 Trp Gly Pro Asn Asp Ala Asn Thr Trp Leu Gly Ser Gln Asn Ala Pro
435
440
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525 Glu Ser Leu Leu Val Arg Asn Arg Thr Ala Ala Trp Asn Gly Ala Gln Page 105

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Pro Gln Tyr Arg Ile Pro Ser Asp Ala Thr Asp Met Tyr Val Tyr Val 610 620
Glu Thr Ala Asp Asp Thr Ile Asn Phe Tyr Ile Asp Glu Ala Ile Gly
625 630 635 640
Ala Val Ala Gly Thr Val Ile Glu Gly Pro Ala Pro Gln Pro Thr Gln
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Pro Pro Val Leu Leu Gly Asp Val Asn Gly Asp Gly Thr Ile Asn Ser 660 665 _ 670
Thr Asp Leu Thr Met Leu Lys Arg Ser Val Leu Arg Ala Ile Thr Leu 675 680 685
Thr Asp Asp Ala Lys Ala Arg Ala Asp Val Asp Lys Asn Gly Ser Ile 690 700
Asn Ser Thr Asp Val Leu Leu Ser Arg Tyr Leu Leu Arg Val Ile
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Asp Lys Phe Pro Val Ala Glu Asn Pro Ser Ser Ser Phe Lys Tyr Glu
725 730 735
Ser Ala Val Gln Tyr Arg Pro Ala Pro Asp Ser Tyr Leu Asn Pro Cys
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745
750
Pro Gln Ala Gly Arg Ile Val Lys Glu Thr Tyr Thr Gly Ile Asn Gly 755 760 765
Thr Lys Ser Leu Asn Val Tyr Leu Pro Tyr Gly Tyr Asp Pro Asn Lys
770
780
Lys Tyr Asn Ile Phe Tyr Leu Met His Gly Gly Gly Glu Asn Glu Asn 785 790 795 800
Thr Ile Phe Ser Asn Asp Val Lys Leu Gln Asn Ile Leu Asp His Ala 805 810 815
Ile Met Asn Gly Glu Leu Glu Pro Leu Ile Val Val Thr Pro Thr Phe 820 825 830
Asn Gly Gly Asn Cys Thr Ala Gln Asn Phe Tyr Gln Glu Phe Arg Gln 835 840 845
Asn Val Ile Pro Phe Val Glu Ser Lys Tyr Ser Thr Tyr Ala Glu Ser
850 855 860
Thr Thr Pro Gln Gly Ile Ala Ala Ser Arg Met His Arg Gly Phe Gly 870 875 880
Gly Phe Ser Met Gly Gly Leu Thr Thr Trp Tyr Val Met Val Asn Cys
885 890 895
Leu Asp Tyr Val Ala Tyr Phe Met Pro Leu Ser Gly Asp Tyr Trp Tyr 900 905 910
Gly Asn Ser Pro Gln Asp Lys Ala Asn Ser Ile Ala Glu Ala Ile Asn 915 920 925
Arg Ser Gly Leu Ser Lys Arg Glu Tyr Phe Val Phe Ala Ala Thr Gly 930 940
Ser Glu Asp Ile Ala Tyr Ala Asn Met Asn Pro Gln Ile Glu Ala Met
945 950 955 960
Lys Ala Leu Pro His Phe Asp Tyr Thr Ser Asp Phe Ser Lys Gly Asn 965 970 975
Phe Tyr Phe Leu Val Ala Pro Gly Ala Thr His Trp Trp Gly Tyr Val
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<223> Obtained from an environmental sample

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accctgcgcg gggtttacga aaaggacttc accatcggcg tggccatgaa cgggggccag
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                                                                                                 1020
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20 25 30
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Ala Ala Thr Ala Glu Glu Glu Ala Thr Leu Arg Gly Val Tyr Glu Lys
     Phe Thr Ile Gly Val Ala Met Asn Gly Gly Gln Ala Ser Gly Arg
Asn Ala Ala Ala Gly Glu Ile Ile Gly Lys Gln Phe Ser Ser Leu Thr 65 70 75 80
Ala Glu Asn Asp Met Lys Trp Gln Met Ile His Pro Gln Glu Gly Gln 85 90 95
Tyr Arg Phe Glu Thr Ser Asp Ala Tyr Val Ala Phe Ala Glu Lys His
100 105
                                                                        110
Lys Met Glu Val Ile Gly His Thr Leu Val Trp His Ser Gln Thr Pro
Gln Trp Val Phe Gln Gly Glu Asn Gly Gln Pro Ala Thr Lys Glu Glu
                                 135
Leu Leu Lys Arg Met Arg Asp His Ile His Ala Val Ala Gly Arg Tyr
145 150 155 160
Lys Gly Lys Ile Lys Gly Trp Asp Val Val Asn Glu Ala Leu Ser Asp
165 170 175
Gly Gly Asp Asp Ile Leu Arg Gln Ser Pro Trp Arg Arg Ile Ile Gly
180 185 190
Asp Asp Phe Ile Asp Tyr Ala Phe Arg Tyr Ala Lys Glu Ala Ala Pro
                                      200
                                                                   205
     Ala Glu Leu Tyr Tyr Asn Asp Tyr Asn Leu Glu Ile Pro Arg Lys
210 215 220
     Ala Asn Cys Ile Thr Leu Val Lys Gly Met Leu Glu Arg Gly Val
     Ile Asp Gly Ile Gly Thr Gln Ser His Phe Gln Leu Gly Phe Pro
Ser Leu Asp Asp Val Glu Ala Thr Ile Lys Glu Phe Ala Ala Leu Gly
260 265 270
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Met Lys val Met Ile Thr Glu Leu Asp Val Asp Val Leu Pro Arg Asn

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Asn Pro Gly Val Ala Asp Ile Ala Asn Arg Glu Gln Gly Ala Asn Pro 290 295 300
     Thr Glu Gly Leu Pro Asp Asp Val Gln Glu Lys Leu Ala Lys Arg
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                                                   315
Tyr Glu Asp Ile Phe Arg Ile Tyr Leu Lys Tyr Arg Asp His Val Thr 325 330 335
Arg Val Thr Phe Trp Gly Leu Asp Asp Gly Met Thr Trp Leu Asn Gly 340 345 350
 Phe Pro Val Arg Gly Arg Thr Asn His Pro Leu Leu Tyr Asp Arg Gln 355 360 365
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Lys Ser His Phe Leu Ile Gly Ala Ala Val Asn Pro Leu Thr Leu Gln
20 25 30
Thr Gln Glu Leu Ile Lys Lys His Phe Asn Ser Ile Thr Ala Glu
Asn Glu Met Lys Phe Glu Glu Leu Gln Pro Glu Pro Gly His Phe Thr
50 55 60
                                                       60
Phe Asp Val Gly Asp Lys Met Val Ala Phe Ala Lys Glu Asn Gly Met 65 70 75 80
Lys Val Arg Gly His Thr Leu Ile Trp His Asn Gln Thr Pro Asp Trp 85 90 95
Met Phe Lys Asn Glu Asp Gly Ser Val Thr Asp Arg Asp Thr Leu Leu
100 105 110
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Glu Arg Met Lys Leu His Ile Thr Thr Val Met Glu His Tyr Lys Gly

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Gln Ile Tyr Cys Trp Asp Val Val Asn Glu Ala Ile Ala Asp Glu Gly
130
140
      130
                                  135
                                                              140
Ser Glu Leu Leu Arg His Ser Lys Trp Thr Glu Ile Ile Gly Asp Asp
145 150 155 160
Phe Ile Glu Lys Ala Phe Glu Tyr Ala His Glu Ala Asp Pro Glu Ala
                       165
                                                   170
Leu Leu Phe Tyr Asn Asp Tyr Asn Glu Ser His Pro His Lys Arg Asp
180 185 190
Lys Ile Tyr Thr Leu Ile Lys Arg Leu Val Asp Lys Gly Ile Pro Ile
195 200 205
His Gly Val Gly Leu Gln Ala His Trp Asn Leu Thr Asp Pro Ser Tyr
210 215 220
Glu Glu Ile Arg Ala Ala Ile Glu Lys Tyr Ala Ser Leu Gly Leu Glu
225 230 235 240
Ile His Leu Thr Glu Met Asp Val Ser Val Phe Asn Phe Glu Asp Arg
245 250 255
Arg Thr Asp Leu Thr Glu Pro Thr Asn Glu Met Lys Thr Leu Gln Val 260 270
Glu Arg Tyr Thr Glu Phe Phe Lys Ile Leu Arg Glu Tyr Ser His Val
275 280 285
Ile Ser Ser Val Thr Phe Trp Gly Ala Ala Asp Asp Tyr Thr Trp Leu 290 295 300
Asp Gly Phe Pro Val Arg Gly Arg Lys Asn Trp Pro Phe Val Phe Asp 305 310 320
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gaggtccacc cagaagcaga ccgctacaac ttcgaaccgt ccgatcgctt cgtcgaattt
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atcaaggaac acattgaaac cgtggtcggc cgatatcgcg gccgcatcca tgcttgggac
gtcgtgaacg aggcaatcga cgacaacggc aaacttcgta gtgggccggt cggagtgccc
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                                                                                                   480
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gaattcgcgc acaccgccga ccccgacgct gaactctatt acaacgacta caacgaatgg
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35 40 45
Gly Thr Asn Gln Val Met Gly Glu Glu Pro Lys Ser Leu Glu Val Val
50 60
Ala Gln Gln Phe Asn Thr Ile Thr Pro Glu Asn Leu Leu Lys Trp Ala
65 70 75 80
Glu Val His Pro Glu Ala Asp Arg Tyr Asn Phe Glu Pro Ser Asp Arg
85 90 95
Phe Val Glu Phe Gly Glu Lys Asn Asn Met Phe Ile Val Gly His Thr
Leu Val Trp His Asn Gln Thr Pro Asp Trp Ala Phe Glu Gly Lys Asp
115 120 125
Gly Lys Pro Leu Asp Arg Glu Thr Ala Leu Ala Arg Île Lys Glu His
130 135
Ile Glu Thr Val Val Gly Arg Tyr Arg Gly Arg Ile His Ala Trp Asp
145 150 155 160
Val Val Asn Glu Ala Ile Asp Asp Asn Gly Lys Leu Arg Ser Gly Pro
165 170 175
Val Gly Val Pro Gly Gln Arg Gly Glu Pro Trp His Ala Ala Ile Gly
180 185 190
Asp Asp Tyr Ile Gln Lys Ala Phe Glu Phe Ala His Thr Ala Asp Pro
195 200 205
Asp Ala Glu Leu Tyr Tyr Asn Asp Tyr Asn Glu Trp His Pro Lys Lys 210 215 220
Ile Glu Ala Ile Ser Gln Leu Val Arg Ser Leu Lys Glu Lys Gly Val
225 230 235 240
Arg Ile Asp Gly Leu Gly Leu Gln Gly His Trp Gly Met Asp Tyr Pro 245 250 255
Lys Val Glu Glu Ile Asp His Met Leu Thr Glu Tyr Gly Lys Leu Gly
260 265 270
Val Lys Leu Met Ile Thr Glu Leu Asp Ile Asn Met Leu Pro Gln Pro
275 280 285
Asp Pro Ser Gln Arg Gly Ala Asp Ile Thr Arg Asn Tyr Glu Leu Arg
290 295 300
Lys Glu Leu Asp Pro Tyr Ser Asp Gly Leu Pro Pro Asp Met Gln Lys 305 310 315 320
Ala Leu Ala Ala Arg Tyr Ala Glu Ile Phe Glu Val Phe Ala Lys His 325 330 335
Arg Asp Lys Leu Asp Arg Val Thr Phe Trp Gly Val His Asp Gly His 340 345
Ser Trp Leu Asn Asn Trp Pro Val Pro Gly Arg Thr Ala Tyr Pro Leu 355 360 365
Leu Phe Asp Thr Lys Leu Gln Pro Lys Pro Ala Phe Asp Ala Val Ile
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                                                                                      240
                                                                                      300
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acgcactata aagggcgtat aaagggctgg gatgtgttga atgaagccat tgaatcggac ggctcctggc gtaaatctcc tttttacgag atattaggcg aagagtacat cccgcttatt
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20 25 30
Gly His Cys Leu Ile Trp His Ser Gln Leu Ala Pro Trp Phe Cys Val
Asp Lys Gln Gly Lys Thr Val Ser Ala Asp Ile Leu Lys Glu Arg Ile
100 105 110
Lys Lys His Ile Gln Thr Ile Val Thr His Tyr Lys Gly Arg Ile Lys
115
120
125
Gly Trp Asp Val Leu Asn Glu Ala Ile Glu Ser Asp Gly Ser Trp Arg
130
135
140
Lys Ser Pro Phe Tyr Glu Ile Leu Gly Glu Glu Tyr Ile Pro Leu Ile
145 150 155 160
Phe Gln Tyr Ala His Glu Ala Asp Pro Glu Ala Glu Leu Tyr Tyr Asn 165 170 175

Asp Tyr Gly Met Asp Gly Lys Ala Lys Arg Asp Lys Val Val Glu Leu 180 185
Val Lys Met Leu Lys Asp Arg Gly Leu Arg Ile Asp Ala Val Gly Met
195 200 205
Gln Gly His Met Gly Met Asp Tyr Pro Ser Val Ser Glu Phe Glu Ala 210 225 220 Ser Ile Leu Ala Phe Ala Ala Ala Gly Val Lys Val Met Val Thr Glu 225 235 240
Trp Asp Met Ser Ala Leu Pro Thr Thr Arg Met Gly Ala Asn Ile Ser 245 250 255
Asp Thr Val Ser Tyr Lys Gln Ser Leu Asn Pro Tyr Pro Asp Gly Leu 260 265 270
Pro Asp Ser Val Ser Val Ala Trp Asn Asn Arg Met Lys
275 280 285
Gly Leu Phe Leu Lys His Ser Asn Ile Ile Thr Arg Val Thr Ala Trp
290 295 300 ____
Gly Val Thr Asp Gly Asp Ser Trp Lys Asn Asn Phe Pro Val Pro Gly 305 310 315 320
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Glu Asn Ile Ile Lys Lys Gln Phe Asn Ser Ile Val Ala Glu Asn Cys
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Thr Leu Lys Thr Leu Tyr Met Tyr Val Glu Ser Pro Asp Pro Thr Leu 175

Glu Tyr Tyr Ile Asp Asp Val Val Val Thr Phe Glu Asn Pro Thr Gln 180

Ile Gly Asn Val Val Ala Asn Gly Thr Phe Glu Asn Glu Asn Thr Ser Page 115

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1200

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165 170 175 Pro Asp Tyr Ile Glu Leu Ala Phe Lys Phe Ala Arg Glu Ala Asp Pro 180 185 190 Asp Ala Lys Leu Phe Tyr Asn Asp Tyr Asn Thr Phe Glu Pro Arg Lys

195 200 205 Asp Ile Ile Tyr Asn Leu Val Lys Asp Leu Lys Glu Lys Gly Leu 210 220 Ile Asp Gly Ile Gly Met Gln Cys His Ile Ser Leu Ala Thr Asp Ile 225 230 240 Lys Gln Ile Glu Glu Ala Ile Lys Lys Phe Ser Thr Ile Pro Gly Ile 245 250 255 Glu Ile His Ile Thr Glu Leu Asp Met Ser Val Tyr Arg Asp Ser Ser 260 265 270 Ser Asn Tyr Pro Glu Ala Pro Arg Thr Ala Leu Ile Glu Gln Ala His Lys Met Met Gln Leu Phe Glu Île Phe Lys Lys Tyr Ser Asn Val Île 290 295 300 Thr Asn Val Thr Phe Trp Gly Leu Lys Asp Asp Tyr Ser Trp Arg Ala 305 310 315 320 Thr Arg Arg Asn Asp Trp Pro Leu Ile Phe Asp Lys Asp His Gln Ala 325 330 335 Lys Leu Ala Tyr Trp Ala Ile Val Ala Pro Glu Val Leu Pro Pro Leu 340 345 350 Pro Lys Glu Ser Arg Ile Ser Glu Gly Glu Ala Val Val Gly Met
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Glu Val Asn Arg Glu Asp Val Gln Val Lys Lys Phe Val Gly Pro Gly
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Gly Lys Trp Tyr Ser Trp Ser Asp Thr Thr Asn Ser Gln Lys Thr Asn 500 510
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20 25 30
Glu Val Gly Leu Lys Asp His Phe Lys Asp His Phe His Ile Gly Thr
Ala Ile Ser Gly Arg Leu Met Thr Glu Met Pro Ala Phe Tyr Arg Asp
                                        55
Leu Val Thr Arg Glu Phe Ser Ala Ile Thr Met Glu Asn Asp Met Lys 65 70 75 80 Trp Glu Arg Leu His Pro Lys Glu Gly Gln Trp Asp Trp Glu Ile Ala 85 90 95
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Asp Lys Phe Val Asn Phe Gly Glu Glu Asn Asp Met Tyr Ile Val Gly
                                              105
His Val Leu Val Trp His Ser Gln Thr Pro Asp Trp Val Phe Gln Asp
                                       120
Ser Arg Gly Lys Pro Ile Ser Arg Asp Ala Leu Leu Lys Arg Met Arg
130 135 140
His Gln Ile Glu Gln Met Ala Gly Arg Tyr Lys Gly Arg Val His Ala
145 _ _ _ 150 _ _ 155 _ _ 160
Trp Asp Val Val Asn Glu Ala Val Asp Glu Asp Gln Gly Trp Arg Lys
165 170 175
Ser Pro Trp Phe Asn Ile Ile Gly Pro Glu Phe Met Glu His Ala Phe
180 185 190
Asn Tyr Ala His Glu Val Asp Pro Asp Ala His Leu Leu Tyr Asn Asp
195 200 205
Tyr Asn Met His Gly Arg Glu Lys Arg Glu Phe Val Leu Asp Phe Ile
210 215 220
Lys Arg Tyr Lys Lys Gly Ile Pro Ile Gln Gly Ile Gly Met Gln 225 230 235 240
Gly His Val Gly Leu Ser Phe Pro Asp Ile Ser Glu Phe Glu Lys Ser 245 250 255
Leu Gln Ala Tyr Ala Lys Gln Gly Met Arg Met His Ile Thr Glu Leu 260 270
Asp Met Asp Val Leu Pro Val Ala Trp Asp His Ile Gly Ala Glu Ile 275 280 285
Ser Thr Glu Phe Asp Tyr Ala Asp Glu Leu Asp Pro Trp Pro Lys Gly 290 _ _ _ 295 _ 300 _ _ _
Leu Pro Glu Glu Val Glu Gln Glu Phe Thr Asp Arg Tyr Thr Ala Phe 305 310 315 320
Phe Lys Leu Phe Leu Lys Tyr Arg Asp Asp Ile Glu Arg Val Thr Phe 325 330 335
Trp Gly Thr Gly Asp Ala Glu Ser Trp Lys Asn Asn Phe Pro Val Arg
340 345 350
Gly Arg Thr Asn Tyr Pro Leu Leu Phe Asp Arg Arg Tyr Arg Arg Lys 355
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180
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agggagttca acatactcac gccagagaac caaatgaagt gggacagcct tcacccagag
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cctgacaggt acaacttcac ttacgcagag cgtcatgtcg agttcgcttt ggaaaacaac atgctcgttc acggccacac actcgtttgg cacaaccaac ttccgttctg gttgaacaga cagtggacca aagaagaact cctgaaagtc cttgaggacc acatcaaaac agtcgtcggt
                                                                                                      300
                                                                                                      360
                                                                                                      420
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                                                                                                      660
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                                                                                                      960
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<213> Unknown

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Leu Pro Asp Ser Asn Lys Tyr Thr Glu Val Ala Lys Arg Glu Phe Asn 50 55 60
Ile Leu Thr Pro Glu Asn Gln Met Lys Trp Asp Ser Leu His Pro Glu 65 70 75 80
Pro Asp Arg Tyr Asn Phe Thr Tyr Ala Glu Arg His Val Glu Phe Ala
85 90 95
Leu Glu Asn Asn Met Leu Val His Gly His Thr Leu Val Trp His Asn 100 105 110
Gln Leu Pro Phe Trp Leu Asn Arg Gln Trp Thr Lys Glu Glu Leu Leu
115 120 125
Lys Val Leu Glu Asp His Ile Lys Thr Val Val Gly His Phe Lys Gly 130 140
Arg Val Lys Ile Trp Asp Val Val Asn Glu Ala Val Ser Asp Met Gly
145 150 155 160
Ser Tyr Arg Glu Thr Ile Trp Tyr Lys Thr Ile Gly Pro Glu Tyr Ile
165 170 175
Glu Lys Ala Phe Val Trp Ala Arg Gln Ala Asp Pro Glu Ala Ile Leu
180 185
                                      185
Ile Tyr Asn Asp Tyr Asn Ile Glu Thr Ile Asn Pro Lys Ser Asn Phe
195 200 205
Thr Tyr Gln Leu Ile Lys Glu Leu Lys Glu Lys Gly Val Pro Ile Asp
210 215 220
Gly Ile Gly Phe Gln Met His Ile Asp Ile Asn Gly Ile Asn Tyr Asp 225 230 235 240
Ser Phe Arg Asn Asn Leu Lys Arg Phe Ala Asp Leu Gly Leu Lys Leu 255
Tyr Ile Thr Glu Met Asp Val Arg Ile Pro Lys Asn Ala Thr Glu Lys 260 270
Asp Leu Asp Arg Gln Ala Glu Ile Tyr Ala Lys Ile Phe Glu Ile Cys 275 280 285
Leu Glu Asn Pro Ala Val Gln Ala Ile Gln Phe Trp Gly Phe Thr Asp
290 295 300
Lys Tyr Ser Trp Val Pro Gly Phe Phe Ser Gly Tyr Asp His Ala Leu 305 310 315 320
Ile Phe Asp Arg Asp Tyr Ser Pro Lys Pro Ala Tyr Phe Ala Ile Lys
325 330 335
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<211> 1131
<212> DNA
<213> Unknown
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                                                                                    120
gccttaagtg ctacacaaat tcagggcaaa gagccgggca cactggaatt ggtaacacag caatttaacg cggtgacggc agaaaacgtg atgaagtggg aaatcattga acctgtggaa ggccagttca actttgctgc cgcgacgcc atgattgaat tcgccgaagc caatcatatc
                                                                                    180
                                                                                    240
                                                                                    300
aaggtgatag gccatgtgct gttatggcac gaacaaacac cagcctgggt atttctggac
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gccaaaggcc aggccgcctc aaaggaactg gtgttatcac ggctaaaaaa ccatatcaat
                                                                                    420
                                            Page 121
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gccgtaatgg gccgctacaa aggccgtatt catggctggg atgcagtcaa cgaagcctta aatgaagacg gcactctgcg ccaatccaac tggtataaag ctttaggcga cgactatata
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gccacagtet ttgaactggc gcatcaggcc gacccgaaag ccgaactcta ttacaacgac
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ttcaatttat ttäaaccgga äaaacgcgct ggtgtactca aactggtggc agctttaaaa
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gcgaaaaatg tgcctatcca cggcataggc gagcaaggcc attacagcct ggattaccct gagctgcagc aagtagaaga ctctattgtg gcttttaaaa acactggcct gaaagtggtg attaccgaac tggatatctc agttttaccc ttccctgagc cagaaaagat tggtgctgat
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gaagtcagcg atcaactgac agaaaaatac ctgcaattat ttcagctatt tttacgccac
                                                                                      960
agcgacgcca tcgaacgcgt gaccttatgg ggcgtaaacg acaaccaaac ctggcgcaac
aactggccaa tgaaaggcag aacagactac cccttactct tcgaccggaa aaaccagcca
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<212> PRT
<213> Unknown
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Ser Lys Asn Phe Ser Ile Gly Thr Ala Leu Ser Ala Thr Gln Ile Gln
35 40 45
Gly Lys Glu Pro Gly Thr Leu Glu Leu Val Thr Gln Gln Phe Asn Ala
50 55 60
Val Thr Ala Glu Asn Val Met Lys Trp Glu Ile Ile Glu Pro Val Glu 65 70 75 80
Gly Gln Phe Asn Phe Ala Ala Ala Asp Ala Met Ile Glu Phe Ala Glu
85 90 95
Ala Asn His Ile Lys Val Ile Gly His Val Leu Leu Trp His Glu Gln
100 105 110
Thr Pro Ala Trp Val Phe Leu Asp Ala Lys Gly Gln Ala Ala Ser Lys
115
120
125
Glu Leu Val Leu Ser Arg Leu Lys Asn His Ile Asn Ala Val Met Gly
     130
                             135
Arg Tyr Lys Gly Arg Ile His Gly Trp Asp Ala Val Asn Glu Ala Leu
Asn Glu Asp Gly Thr Leu Arg Gln Ser Asn Trp Tyr Lys Ala Leu Gly
                   165
                                            170
Asp Asp Tyr Ile Ala Thr Val Phe Glu Leu Ala His Gln Ala Asp Pro
180 185 190
Lys Ala Glu Leu Tyr Tyr Asn Asp Phe Asn Leu Phe Lys Pro Glu Lys 195 200 205
Arg Ala Gly Val Leu Lys Leu Val Ala Ala Leu Lys Ala Lys Asn Val 210 _ _ _ 215 _ _ 220
Pro Ile His Gly Ile Gly Glu Gln Gly His Tyr Ser Leu Asp Tyr Pro 235 240
Glu Leu Gln Gln Val Glu Asp Ser Ile Val Ala Phe Lys Asn Thr Gly
245 250 255
Leu Lys Val Val Ile Thr Glu Leu Asp Ile Ser Val Leu Pro Phe Pro 260 _ 265 270
Glu Pro Glu Lys Ile Gly Ala Asp Ile Ser Leu Asn Met Gln Leu Lys
Gln Glu Leu Asn Pro Tyr Ala Asp Gly Leu Pro Lys Glu Val Ser Asp
                             295
Gln Leu Thr Glu Lys Tyr Leu Gln Leu Phe Gln Leu Phe Leu Arg His
305 310 315 320
Ser Asp Ala Ile Glu Arg Val Thr Leu Trp Gly Val Asn Asp Asn Gln 325 330 335
Thr Trp Arg Asn Asn Trp Pro Met Lys Gly Arg Thr Asp 340
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ggaacggtca attictgtat gtatgccaac ggccgttaca cgtctaactg gaacggcatc aacaattggg tcggcggcaa aggctggcaa accggctcgc gcagaaacgt cacctactct ggctcgttca actctcccgg caatggctat ctggctgctc tactggctgg accaccaatc
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gcacgcggct tgaacctcgg cacgcacaac taccaagtga tggcgaccga gggatatcag
agcaacggca gctccgacat caccattagc gacaacccgg gaccgacgcc aggacccact ccgaacccga atcccacgcc gggcaccaag aatttcacgg tgcgcgcgcg cggaaccgcg gggggtgagt ccatcacgct gcgtgtgaac aatcagaacg tgcagacctg gacgctgtcg accagctacc agaacttcac ggcgtccacg acgttgagtg gtggcatcac ggtcgcgttc
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                                                                                                   720
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                                                                                                  840
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<212> PRT
<213> Unknown
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20 25 30
Phe Ser Phe Trp Lys Asp Asn Pro Gly Thr Val Asn Phe Cys Met Tyr 35 40 45
Ala Asn Gly Arg Tyr Thr Ser Asn Trp Asn Gly Ile Asn Asn Trp Val
Gly Gly Lys Gly Trp Gln Thr Gly Ser Arg Arg Asn Val Thr Tyr Ser
65 70 75 80
Gly Ser Phe Asn Ser Pro Gly Asn Gly Tyr Leu Ala Ala Leu Leu Ala
85 90 95
Gly Pro Pro Ile Leu Leu Val Glu Tyr Tyr Ile Ile Glu Ser Trp Gly
100 105 110
Asn Trp Arg Pro Pro Gly Ser Asp Gly Thr Leu Leu Gly Thr Val Thr 115 120 125
Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Arg Ser Arg Arg Thr Asn Ala
130 135 140
Pro Cys Ile Thr Gly Asn Ser Cys Asn Phe Asp Gln Tyr Trp Ser Val
Arg Gln Ser Lys Arg Val Gly Gly Thr Ile Thr Thr Gly Asn His Phe
165 170 175
Asp Ala Trp Ala Ala Arg Gly Leu Asn Leu Gly Thr His Asn Tyr Gln
180 _____ 185 ____ 190 ____
Val Met Ala Thr Glu Gly Tyr Gln Ser Asn Gly Ser Ser Asp Ile Thr
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200
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Ile Ser Asp Asn Pro Gly Pro Thr Pro Gly Pro Thr Pro Asn Pro Asn 210 220
Pro Thr Pro Gly Thr Lys Asn Phe Thr Val Arg Ala Arg Gly Thr Ala
225 230 235 240
Gly Gly Glu Ser Ile Thr Leu Arg Val Asn Asn Gln Asn Val Gln Thr
                                                                  250
Trp Thr Leu Ser Thr Ser Tyr Gln Asn Phe Thr Ala Ser Thr Thr Leu 260 265 270
                                                          265
Ser Gly Gly Ile Thr Val Ala Phe Thr Asn Asp Gly Gly Ser Arg Asp 285
Val Gln Val Asp Tyr Ile Gln Val Asn Gly Ala Thr Arg Gln Ser Glu
290 295 300
Ser Gln Thr Tyr Asn Thr Gly Leu Tyr Ala Asn Gly Ser Cys Gly Gly 305 310 315 320
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Asn Thr Pro
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<213> Unknown
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                                                                                                                                  60
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                                                                                                                                180
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ccgaatggag gaactttcga tgctacctgg aatggcaccg agaatatcct ggctagagct ggtaagaaat ggggctcgtc cagcactacc accccacgt ccgcaggcaa tattactctt gaattcgcgg cgacatggtc ctcaagcgat aacgtaaaaa tgcttggagt ctatggctgg gcgtactatc caactggaag tatcccgact aaacaggaaa atggagcaag tacctcattc acaaatcaaa ttgagtacta catcatccag gatcgtggta gctataatgc tgcatcgggt ggaacgaact ccaaaaata cggcgaaggg acgatcgatg gaattctgta tgaattctat atcgcaggac gaatcaacca gcctgatctg tcaggaaaga gtggaaactt caagcaatac ttcagcgtcc cgaaaagtac gagcagccat aggcaaagtg ggacgattac cgtttccaaa cattccagg cctgggaaaa tgccggaatg aaaatgatgt cctgtcgctt gtatgaagtc gcaatgaaag tcgagtccta taccggttct gcaggaagt tcgagtcaatga cgttacaag cattccag
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                                                                                                                                360
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                                                                                                                                540
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                                                                                                                              1140
                                                                                                                              1200
tccgcagcaa cgacgagtgt cagcaatgga atcgcgactg tcaatgtgac caccattgga
                                                                                                                              1260
tctcaaacct atcaaccca gctaattcag tataacgtgg ctctttacaa ggatatgagc tacaagctca ccttcaaggc aaaagctgct gctgcaagga aaattgaagt cgcattccaa cagtcggtgg acccatgggc tggatatgct tccaaggaat tcgatcttac aacgacagag cagacatatg agttcgtatt taaaatgact agcgctactg acaggcgtt acagttcgcg ttcaattgg gccaggcaat aggacagacatatatacg
                                                                                                                              1320
                                                                                                                              1380
                                                                                                                              1500
                                                                                                                              1560
acagctggta caacacccgt attccgtgga tataatgagg cggcaacaca ggagaggcct gtattcatat ccttggatgg taggacgttg aacattgttc cagtgtatgg agccaaactg
                                                                                                                              1620
                                                                                                                              1680
caggicaagi tagiggacai caaiggiaag aigagagcci cciicaaigi ggicggaati
                                                                                                                              1740
gcttccatcc cgctgtccaa tatccccgct gggcggtatt atattgacgt aagtggtgac
                                                                                                                              1800
ggcgttaagc aggcatcccc gatagttctg gaataa
                                                                                                                              1836
<210> 156
<211> 611
<212> PRT
<213> Unknown
<223> Obtained from an environmental sample
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<221> SIGNAL <222> (1)...(21)

<400> 156 Met Lys Gly Leu Ile Ala Ala Ala Leu Ala Gly Leu Ala Phe Gly Ala 1 10 15 10 Ser Leu Ser Trp Gly Gln Cys Thr Thr Phe Thr Thr Ser Thr Ile Gln
20 25 30 Asn Cys Asn Gly Ile Asp Tyr Glu Leu Trp Ser Gln Asn Asn Lys Gly
35 40 45 Thr Val Ser Met Lys Ile Thr Gly Gly Ser Thr Asn Pro Asn Gly Gly 50 55 60 Thr Phe Asp Ala Thr Trp Asn Gly Thr Glu Asn Ile Leu Ala Arg Ala 65 70 75 80 Gly Lys Lys Trp Gly Ser Ser Ser Thr Thr Pro Thr Ser Ala Gly
85
90
95 Asn Ile Thr Leu Glu Phe Ala Ala Thr Trp Ser Ser Ser Asp Asn Val 100 105 110 Lys Met Leu Gly Val Tyr Gly Trp Ala Tyr Tyr Pro Thr Gly Ser Ile 115 120 125 115 Pro Thr Lys Gln Glu Asn Gly Ala Ser Thr Ser Phe Thr Asn Gln Ile 130 140 Glu Tyr Tyr Ile Ile Gln Asp Arg Gly Ser Tyr Asn Ala Ala Ser Gly 145 150 160 Gly Thr Asn Ser Lys Lys Tyr Gly Glu Gly Thr Ile Asp Gly Ile Leu 165 170 175 Tyr Glu Phe Tyr Ile Ala Asp Arg Ile Asn Gln Pro Asp Leu Ser Gly
180 185 190 Lys Ser Gly Asn Phe Lys Gln Tyr Phe Ser Val Pro Lys Ser Thr Ser 195 200 205 Ser His Arg Gln Ser Gly Thr Ile Thr Val Ser Lys His Phe Gln Ala 210 215 220 Trp Glu Asn Ala Gly Met Lys Met Met Ser Cys Arg Leu Tyr Glu Val 225 230 235 240 Ala Met Lys Val Glu Ser Tyr Thr Gly Ser Ala Thr Gly Val Gly Ser 245 250 255 Ala Lys Val Thr Lys Asn Ile Leu Thr Ile Gly Gly Ile Leu Ser Ser 260 265 270 . Ser Ser Thr Ala Ser Ser Ser Ser Thr Val Ser Ser Ser Ser Ser Asn 275 280 285 Ala Tyr Thr Leu Val Thr Asn Val Ser Pro Ala Gly Ala Gly Thr Val 290 295 300 Thr Arg Ser Pro Asn Thr Ala Thr Tyr Ala Pro Asn Ala Ser Val Gln 305 310 315 320 Leu Thr Ala Thr Pro Ser Thr Gly Trp Lys Phe Val Gly Trp Ala Gly 325 330 335 Asp Leu Thr Ser Thr Thr Ser Thr Ala Thr Val Thr Met Thr Lys Asp 340 345 350 Ile Thr Ala Thr Ala Lys Phe Glu Leu Val Ser Gly Asp Gly Thr Thr 355 360 365 Asn Leu Ile Lys Asp Gly Asn Phe Pro Ser Ser Val Ile Ser Thr 370 375 380 Gly Asp Gly Thr Ser Trp Lys Leu Gly Gln Gly Thr Asn Trp Gly Asn 385 395 400 Ser Ala Ala Thr Thr Ser Val Ser Asn Gly Ile Ala Thr Val Asn Val 405 410 415 Thr Thr Ile Gly Ser Gln Thr Tyr Gln Pro Gln Leu Ile Gln Tyr Asn
420 425 430 Val Ala Leu Tyr Lys Asp Met Ser Tyr Lys Leu Thr Phe Lys Ala Lys 435 440 445 435 Ala Ala Ala Arg Lys Ile Glu Val Ala Phe Gln Gln Ser Val Asp 450 455 460 Pro Trp Ala Gly Tyr Ala Ser Lys Glu Phe Asp Leu Thr Thr Thr Glu 465 470 480 Gin Thr Tyr Glu Phe Val Phe Lys Met Thr Ser Ala Thr Asp Thr Ala 485 490 495 Ser Gln Phe Ala Phe Asn Leu Gly Gln Ala Thr Gly Ala Val Asn Ile 500 505 510 Ser Asp Val Lys Leu Val Tyr Thr Thr Ala Gly Thr Thr Pro Val Phe Page 125

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Arg Gly Tyr Asn Glu Ala Ala Thr Gln Glu Arg Pro Val Phe Ile Ser 530 540
Leu Asp Gly Arg Thr Leu Asn Ile Val Pro Val Tyr Gly Ala Lys Leu 545 550 560
Gln Val Lys Leu Val Asp Ile Asn Gly Lys Met Arg Ala Ser Phe Asn 575
Val Val Gly Ile Ala Ser Ile Pro Leu Ser Asn Ile Pro Ala Gly Arg
580
585
590
Tyr Tyr Ile Asp Val Ser Gly Asp Gly Val Lys Gln Ala Ser Pro Ile
595 600 605
Val Leu Glu
     610
<210> 157
<211> 645
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 157
atgtttaagt taagtaagaa aattttgatg gtgttattaa caatttcaat gagttttatt agcttatttg cagtaaccgc gtatgcagct tcgacagact actggcaaaa ttggactgat ggtggtggga cagtaaatgc taccaatgga tctgatggca attacagtgt ttcatggtca aattgcggga attttgtt tggtaaaggc tggactaccg gatcagcaac tagggtaata aactataaatg ccggagtctt ttcgccgtc ggcaatgcgt atttagctct ttatgggtga
                                                                                                 60
                                                                                               120
                                                                                               180
                                                                                               240
                                                                                               300
acgagaaatt cactcataga atattacgtc gttgatagct ggggggactta tagacctact ggaacttata aaggcactgt gactagtgat ggagggacat atgacatata cacgactaca cgaaccaacg caccttccat tgacggcaat aatacaaatt tcacccagtt ctggagtgtt
                                                                                               360
                                                                                               420
                                                                                               480
aggcagtcaa agagaccgat tggtaccaac aataccatca cttttagcaa ccacgttaac
                                                                                               540
gcctggaaga gtaaaggaat gaatctgggg agtagttggg cttatcaggt attagcgaca
                                                                                               600
gagggatatc aaagtagtgg gtactctaac gtaacggtct ggtaa
                                                                                               645
<210> 158
<211> 214
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(29)
<400> 158
Met Phe Lys Leu Ser Lys Lys Ile Leu Met Val Leu Leu Thr Ile Ser
1 10 15
Met Ser Phe Ile Ser Leu Phe Ala Val Thr Ala Tyr Ala Ala Ser Thr
Asp Tyr Trp Gln Asn Trp Thr Asp Gly Gly Gly Thr Val Asn Ala Thr
Asn Gly Ser Asp Gly Asn Tyr Ser Val Ser Trp Ser Asn Cys Gly Asn 50 55 60
Phe Val Val Gly Lys Gly Trp Thr Thr Gly Ser Ala Thr Arg Val Ile
65 70 75 80
Asn Tyr Asn Ala Gly Ala Phe Ser Pro Ser Gly Asn Gly Tyr Leu Ala
85 90 95
Leu Tyr Gly Trp Thr Arg Asn Ser Leu Ile Glu Tyr Tyr Val Val Asp
100 105 110
Ser Trp Gly Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Thr Val Thr
115 120 125
Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Thr Thr Thr Arg Thr Asn Ala
130 135 140
Pro Ser Ile Asp Gly Asn Asn Thr Asn Phe Thr Gln Phe Trp Ser Val
145 150 155 160
Arg Gln Ser Lys Arg Pro Ile Gly Thr Asn Asn Thr Ile Thr Phe Ser
```

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Asn His Val Asn Ala Trp Lys Ser Lys Gly Met Asn Leu Gly Ser Ser
180 185 190
Trp Ala Tyr Gln Val Leu Ala Thr Glu Gly Tyr Gln Ser Ser Gly Tyr
Ser Asn Val Thr Val Trp
<210> 159
<211> 1041
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample
<400> 159
atgatcagtc tcaaacgagt ggcggcgctc ctgtgcgtcg caggtctggg catgtctgcg gcaaacgcgc agacctgcct cacgtcgagt caaaccggca ctaacaatgg cttctattat
                                                                                          60
                                                                                         120
tccttctgga aggacagtcc gggcacggtg aatttttgcc tgcagtccgg cggccgttac
                                                                                         180
acatcgaact ggagcggcat caacaactgg gtgggcggca agggatggca gaccggttca cgccggaaca tcacgtactc gggcagcttc aattcaccgg gcaacggcta cctggcgctt tacggatgga ccaccaatcc actcgtcgag tactacgtcg tcgatagctg ggggagctgg
                                                                                         240
                                                                                         300
                                                                                         360
cgtccgccgg gttcggacgg aacgttcctg gggacggtca acagcgatgg cggaacgtat
                                                                                         420
gacatctatc gcgcgcagcg ggtcaacgcg ccgtccatca tcggcaacgc cacgttctat
                                                                                         480
540
                                                                                         600
                                                                                         660
                                                                                         720
                                                                                         780
                                                                                         840
                                                                                         900
atcgtcgtga acggccagac gcgccagtcc gaagcccaga gctacaacac cggcctttat
                                                                                         960
                                                                                        1020
gcgaacgggc gttgcggcgg tggctccaac agcgaatgga tgcattgcaa cggcgccatc
ggctacggca atacaccgta a
                                                                                        1041
<210> 160
<211> 346
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(23)
<400> 160
Met Ile Ser Leu Lys Arg Val Ala Ala Leu Leu Cys Val Ala Gly Leu 1 5 10 15 Gly Met Ser Ala Ala Asn Ala Gln Thr Cys Leu Thr Ser Ser Gln Thr 20 25 30
Gly Thr Asn Asn Gly Phe Tyr Tyr Ser Phe Trp Lys Asp Ser Pro Gly
35 40 45
Thr Val Asn Phe Cys Leu Gln Ser Gly Gly Arg Tyr Thr Ser Asn Trp 50 55 60
Ser Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Gln Thr Gly Ser 65 70 75 80
Arg Arg Asn Ile Thr Tyr Ser Gly Ser Phe Asn Ser Pro Gly Asn Gly
85
90
95
Tyr Leu Ala Leu Tyr Gly Trp Thr Thr Asn Pro Leu Val Glu Tyr Tyr
100 105 110
Val Val Asp Ser Trp Gly Ser Trp Arg Pro Pro Gly Ser Asp Gly Thr
115 120 125
Phe Leu Gly Thr Val Asn Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Arg
130 140
Ala Gln Arg Val Asn Ala Pro Ser Ile Ile Gly Asn Ala Thr Phe Tyr
145 150 160
Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Val Gly Gly Thr Ile Thr
165 170 175
```

```
Thr Gly Asn His Phe Asp Ala Trp Ala Ser Val Gly Leu Asn Leu Gly
                       180
                                                            185
Thr His Asn Tyr Gln Ile Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly
               195
                                                    200
                                                                                         205
Ser Ser Asp Ile Thr Val Ser Glu Gly Gly Ser Ser Gly Gly Gly
                                             215
                                                                                  220
Ser Ser Thr Ser Ser Ser Ser Gly Gly Gly Thr Lys Ser Phe Thr 225 230 240
Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Ser Ile Thr Leu Arg Val
245 250 255
Asn Asn Gln Asn Val Gln Thr Trp Thr Leu Gly Thr Ser Met Thr Asn 260 265 270
Tyr Thr Ala Ser Thr Ser Leu Ser Gly Gly Ile Thr Val Val Tyr Thr
                                                    280
Asn Asp Ser Gly Asn Arg Asp Val Gln Val Asp Tyr Ile Val Val Asn
290 295 300
Gly Gln Thr Arg Gln Ser Glu Ala Gln Ser Tyr Asn Thr Gly Leu Tyr 305 310 320
Ala Asn Gly Arg Cys Gly Gly Gly Ser Asn Ser Glu Trp Met His Cys 325 330 335
Asn Gly Ala Ile Gly Tyr Gly Asn Thr Pro
340 345
 <210> 161
 <211> 1047
 <212> DNA
 <213> Unknown
<223> Obtained from an environmental sample
<400> 161
atgitcaaag gictittgaa atcggiccic accggcaagc gagccggigc ggigticatc
tgtctggccg gactgtggat gacacaggcg caggcgcaga cgtgcatcgg ttcaccacaa acgggcaaca acggcggctt cttcttttcg ttctggaaag acaatccggg gtcggtgaat ttctgcatgt actccggcgg tcgctatacc tccagctgga gcggcatcaa caactgggta
                                                                                                                                  120
                                                                                                                                  180
                                                                                                                                  240
ggtgggaagg gctggcaaac cggttcatcc cgcacggtga gcggcatcaa caactgggta ggtgggaagg gctggcaaac cggttcatcc cgcacggtga cgtattcggg cacgttcaac tcgccgggaa acggctacct gactctgtac ggatggacca ccaatccgct ggtcgagtac tacatcgtgg acagctgggg cagctaccgt ccgcctggag gccagggctt catggggcacg gtcaccagcg acggcggaac gtatgacatc taccgggttc gccgcaccaa tgcgccgtgc atcacaggca acaactgcaa cttcgaccag tactggagcg gtaggcacca tcaccacgc caaccattc aacgcgtggc gtacgctcgg caggcgggtg ggcggacca actaccaggt gatggcgacc gaaggattcc agagcagtgg cagctcggac atcaccgtga gcgaaggatc tggcggtggc ggcgaggtg gcggcggtgg caccaagagc ttcacggtgc gcgcgcgcg caccgcgggc ggcgaggtca tcaccgtga caccacggac caccacggtg ggcgaggtca tcaccgtga gcgcgcgcg caccacgggc ggcgaggtca tcaccacgc gaaggatcca tcacgctgcg caccaagagc ttcacggtgc gcgcgcgcg caccgcggc ggcgagtcca tcaccacgc cgtcaacaac caggtcgtgc agagctggac cttgagcacc agcatgcaga actacacggc ctcgaccacg ataaaccacgc qcatcacqqt qaacttcacc aacqacggca ccaaccgcga cgtgcaggtg
                                                                                                                                  300
                                                                                                                                  360
                                                                                                                                  420
                                                                                                                                  480
                                                                                                                                  540
                                                                                                                                  600
                                                                                                                                  660
                                                                                                                                  720
                                                                                                                                  780
                                                                                                                                  840
atgagcggcg gcatcacggt gaacttcacc aacgacggca ccaaccgcga cgtgcaggtg
                                                                                                                                  900
gactacatca tegtgaatgg ceagaegegt cagteegaag egeagaegta caacaeeggg
etgtaegeea aeggeegttg eggtggeggg tegaaeageg agtggatgea ttgeaatgge
                                                                                                                                  960
                                                                                                                                1020
gcgatcgggt acggcgacac gccctga
                                                                                                                                1047
<210> 162
<211> 348
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(32)
<400> 162
Met Phe Lys Gly Leu Leu Lys Ser Val Leu Thr Gly Lys Arg Ala Gly
                                                                  10
Ala Val Phe Ile Cys Leu Ala Gly Leu Trp Met Thr Gln Ala Gln Ala 20 25 30 Gln Thr Cys Ile Gly Ser Pro Gln Thr Gly Asn Asn Gly Gly Phe Phe 35 40
```

```
Phe Ser Phe Trp Lys Asp Asn Pro Gly Ser Val Asn Phe Cys Met Tyr 50 _ 60
 Ser Gly Gly Arg Tyr Thr Ser Ser Trp Ser Gly Ile Asn Asn Trp Val
65 70 75 80
 Gly Gly Lys Gly Trp Gln Thr Gly Ser Ser Arg Thr Val Thr Tyr Ser
85 90 95
 Gly Thr Phe Asn Ser Pro Gly Asn Gly Tyr Leu Thr Leu Tyr Gly Trp
 Thr Thr Asn Pro Leu Val Glu Tyr Tyr Ile Val Asp Ser Trp Gly Ser
115 120 125
 Tyr Arg Pro Pro Gly Gly Gln Gly Phe Met Gly Thr Val Thr Ser Asp 130 140
 Gly Gly Thr Tyr Asp Ile Tyr Arg Val Arg Arg Thr Asn Ala Pro Cys
145
150
160
 Ile Thr Gly Asn Asn Cys Asn Phe Asp Gln Tyr Trp Ser Val Arg Gln
165 170 175
 Ser Arg Arg Val Gly Gly Thr Ile Thr Thr Ala Asn His Phe Asn Ala 180 185 190
 Trp Arg Thr Leu Gly Met Asn Leu Gly Gln His Asn Tyr Gln Val Met
195 200 205
 Glu Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Thr Lys Ser
 Phe Thr Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Ser Ile Thr Leu 245 250 255
 Arg Val Asn Asn Gln Val Val Gln Ser Trp Thr Leu Ser Thr Ser Met 260 265 270
 Gln Asn Tyr Thr Ala Ser Thr Thr Met Ser Gly Gly Ile Thr Val Asn 275 280 285
 Phe Thr Asn Asp Gly Thr Asn Arg Asp Val Gln Val Asp Tyr Ile Ile 290 295 300
Val Asn Gly Gln Thr Arg Gln Ser Glu Ala Gln Thr Tyr Asn Thr Gly 305 310 315 320 Leu Tyr Ala Asn Gly Arg Cys Gly Gly Gly Ser Asn Ser Glu Trp Met 325 330 335
His Cys Asn Gly Ala Ile Gly Tyr Gly Asp Thr Pro
 <210> 163
 <211> 1068
 <212> DNA
 <213> Unknown
 <220>
 <223> Obtained from an environmental sample
 <400> 163
 atgaaagcaa agagaatgaa gttgtttgcc gcatttttac tctgttttac gcttgcactt
                                                                                                                   60
 cctggggcag tgcatgcgca gacgatcacc agcaattcgg tcggtacgca tgacggttat
                                                                                                                  120
gactatgaat actggaagga cagcgggaat ggaactatgg ttctcggtag tggcggtacg ttcagtgccg agtggagcaa tatcaataat attctgttcc gtaaaggcaa gaagttcaat gagacgcaga cccatcagca aattggaaca attccataa cctatggtgc cacctaccaa
                                                                                                                  180
                                                                                                                  240
                                                                                                                  300
ccgaatggca attcgtattt aacggtctat ggctggacgg ttgacccct cgtcgaatat
tacattgtcg atagctgggg cagctggcgt ccgcctggag catcgccaaa ggggactgtt
                                                                                                                  360
                                                                                                                  420
aacgttgacg gaggaacgta tgacatttat gagacaactc gtgtcaacca gccttccatt
aaaggcacgg caaccttcaa gcagtattgg agtgtccgga cgtcaaaacg gacgagcgga
accatatctg taagcgagca ctttaaggcc tgggagaaat tgggatgac catgggcaag
                                                                                                                  480
                                                                                                                  540
                                                                                                                  600
accatatety taagegagea etttaaggee tgggagaaat tggggatgae catgggeaag atgtatgaag tegegettae ggttgaagge tateaaagea gtggaagege taatgtgtat agecatacae tgaegategg egggggaaca acacetecae caaceaegg cacaaagate gaageeget tgtatgeeaa aageggaeaa tacaetggga atateagete geegtteaae ggggtegett tgtatgeeaa caatgattee gtgaaattea egeataatt caegaeegge acceataaet teteaeteeg gggggeatea aacaaeteea atatggeeeg ggttgaeetg aaaateggeeg ggeagaegaa ggggaeette tatteeggeg gaageageee tgeggtetat aetetegaata atgteageea tggaaeegga aateaagagg ttgaaetegt tgtaaeegg gataaeeggaa catgggatge ttteattgat tatetegaga tecattaa
                                                                                                                  660
                                                                                                                  720
                                                                                                                  780
                                                                                                                  840
                                                                                                                  900
                                                                                                                  960
                                                                                                                1020
```

<210> 164 <211> 355

1068

<212> PRT <213> Unknown

```
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(26)
<400> 164
Met Lys Ala Lys Arg Met Lys Leu Phe Ala Ala Phe Leu Leu Cys Phe 1 5 10 15
Thr Leu Ala Leu Pro Gly Ala Val His Ala Gln Thr Ile Thr Ser Asn
Ser Val Gly Thr His Asp Gly Tyr Asp Tyr Glu Tyr Trp Lys Asp Ser
35 40 45
Gly Asn Gly Thr Met Val Leu Gly Ser Gly Gly Thr Phe Ser Ala Glu 50 55 60
Trp Ser Asn Ile Asn Asn Ile Leu Phe Arg Lys Gly Lys Lys Phe Asn 65 70 75 80
Glu Thr Gln Thr His Gln Gln Ile Gly Asn Ile Ser Ile Thr Tyr Gly
85 90 95
Ala Thr Tyr Gln Pro Asn Gly Asn Ser Tyr Leu Thr Val Tyr Gly Trp
Thr Val Asp Pro Leu Val Glu Tyr Tyr Ile Val Asp Ser Trp Gly Ser
115 120 125
Trp Arg Pro Pro Gly Ala Ser Pro Lys Gly Thr Val Asn Val Asp Gly 130 135 140
Gly Thr Tyr Asp Ile Tyr Glu Thr Thr Arg Val Asn Gln Pro Ser Ile
145 150 155 160
Lys Gly Thr Ala Thr Phe Lys Gln Tyr Trp Ser Val Arg Thr Ser Lys
165 170 175
Arg Thr Ser Gly Thr Ile Ser Val Ser Glu His Phe Lys Ala Trp Glu
180 185 190
Lys Leu Gly Met Thr Met Gly Lys Met Tyr Glu Val Ala Leu Thr Val
195 200 205
Glu Gly Tyr Gln Ser Ser Gly Ser Ala Asn Val Tyr Ser His Thr Leu
210 225 220
Thr Ile Gly Gly Gly Thr Thr Pro Pro Pro Thr Thr Gly Thr Lys Ile 225 230 235 240
Glu Ala Glu Ser Met Thr Lys Ser Gly Gln Tyr Thr Gly Asn Ile Ser
245 250 255
Ser Pro Phe Asn Gly Val Ala Leu Tyr Ala Asn Asn Asp Ser Val Lys
260 265 270
Phe Thr His Asn Phe Thr Thr Gly Thr His Asn Phe Ser Leu Arg Gly 275 280 285
Ala Ser Asn Asn Ser Asn Met Ala Arg Val Asp Leu Lys Ile Gly Gly 290 295 300
Gln Thr Lys Gly Thr Phe Tyr Phe Gly Gly Ser Ser Pro Ala Val Tyr
305 310 315 320
Thr Leu Asn Asn Val Ser His Gly Thr Gly Asn Gln Glu Val Glu Leu
325 330 335
                  325
Val Val Thr Ala Asp Asn Gly Thr Trp Asp Ala Phe Ile Asp Tyr Leu
340 345 350
Glu Ile His
         355
<210> 165
<211> 1047
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample
<400> 165
gtggggcgca ggagcgccgc cacggcattc atcggcctgg cagcgctgtg tgcctcggcc
                                                                                 60
gccaacgcgc agacctgtct gagctcgagt cagaccggca ccaacaacgg cttctactat
                                                                                120
tcgttctgga ccgacggcgg tggctccgtg cagttctgcc tgcaatccgc cgggcgCtac
                                                                                180
                                          Page 130
```

```
acctccagct ggagcaatgt cggaaactgg gtcggtggca agggctggca gaccggcgcgcgcgcgcgcaaca tcaactattc cggcagcttc aatccctcgg gtaacgcgta cctggccgtc tatggctgga ccacgaatcc cctggtggag tactacatcg tcgacaactg gggtacctat cgtccaccgg gtgggcagg
                                                                                           240
                                                                                           300
                                                                                           360
                                                                                           420
gtctaccgca cgcaacgggt caacgcgccc tccattcagg gcaacgcgac cttctaccag
                                                                                           480
tactggagcg ttcgccagtc gaagcgcacc ggtggaacca tctccaccgg caaccatttc
                                                                                           540
gacggctggg cgacgttcgg catgaacctg ggaaccttca attaccagat cgtggcgacc gagggctacc agagcagcgg caattccgac atcacggtga gcgatggcgg cagcagctcc tcgtcctcca gcagcagcag ttcgtcgtcc tccagcagcg gcggtggcgg caccaagagc
                                                                                           600
                                                                                           660
                                                                                           720
ttcacggtgc gcgcgcggg cacggccgga ggcgagtcga tcagcctgcg ggtcaacaac accaacgtgc agacctggtc gctgaccacc agctaccaga atctcacggc ctcgaccacg
                                                                                           780
                                                                                           840
ctgaccggcg gcatcaccgt caactacacc aacgacagca gcggtcacga cgtacaggtg gactacatca tcgtgaacgg ccagaccgc cagtccgagg cgcagagcta caacaccgga
                                                                                           900
                                                                                           960
čtctatgcca acgggcgctg cggtggtggt ggctacagcg agtggatgca ttgcaacggc
                                                                                          1020
gccatcggct acggcaatac gccgtaa
                                                                                          1047
<210> 166
<211> 348
<212> PRT
<213> Unknown
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(23)
<400> 166
Val Gly Arg Arg Ser Ala Ala Thr Ala Phe Ile Gly Leu Ala Ala Leu
1 _ 5 _ 10 _ 15
Cys Ala Ser Ala Ala Asn Ala Gln Thr Cys Leu Ser Ser Gln Thr
20 25 30
Gly Thr Asn Asn Gly Phe Tyr Tyr Ser Phe Trp Thr Asp Gly Gly Gly 35 40 45
Ser Val Gln Phe Cys Leu Gln Ser Ala Gly Arg Tyr Thr Ser Ser Trp 50 55 60
Ser Asn Val Gly Asn Trp Val Gly Gly Lys Gly Trp Gln Thr Gly Ala 70 75 80
Arg Arg Asn Ile Asn Tyr Ser Gly Ser Phe Asn Pro Ser Gly Asn Ala
Tyr Leu Ala Val Tyr Gly Trp Thr Thr Asn Pro Leu Val Glu Tyr Tyr
100 105 110
Ile Val Asp Asn Trp Gly Thr Tyr Arg Pro Pro Gly Gly Gln Gly Phe
115
120
125
Met Gly Thr Val Val Ser Asp Gly Gly Thr Tyr Asp Val Tyr Arg Thr
130 140
Gln Arg Val Asn Ala Pro Ser Ile Gln Gly Asn Ala Thr Phe Tyr Gln
150
155
160
Tyr Trp Ser Val Arg Gln Ser Lys Arg Thr Gly Gly Thr Ile Ser Thr
165 170 175
Gly Asn His Phe Asp Gly Trp Ala Thr Phe Gly Met Asn Leu Gly Thr
Phe Asn Tyr Gln Ile Val Ala Thr Glu Gly Tyr Gln Ser Ser Gly Asn 195 200 205
Ser Ser Ser Ser Ser Ser Ser Gly Gly Gly Thr Lys Ser 230 235 240
Phe Thr Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Ser Ile Ser Leu 245 250 255
Arg Val Asn Asn Thr Asn Val Gln Thr Trp Ser Leu Thr Thr Ser Tyr 260 265 270
Gln Asn Leu Thr Ala Ser Thr Thr Leu Thr Gly Gly Ile Thr Val Asn
275 280 285
Tyr Thr Asn Asp Ser Ser Gly His Asp Val Gln Val Asp Tyr Ile Ile
290 295 300
Val Asn Gly Gln Thr Arg Gln Ser Glu Ala Gln Ser Tyr Asn Thr Gly 305 310 315 320
```

Leu Tyr Ala Asn Gly Arg Cys Gly Gly Gly Gly Tyr Ser Glu Trp Met

```
335
His Cys Asn Gly Ala Ile Gly Tyr Gly Asn Thr Pro
340 345
<210> 167
<211> 669
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 167
gtgaagctga aaagactgtt caagatcgga ctgctgccgg ccgtattgtt gtttagtgca
                                                                                       60
acgcagcagt taaccgcgca aaccatctgc agcaaccaga ccggcaccaa caacggctac
                                                                                      120
ttctactcgt tctggaagga caccgggtcg gcgtgcatga cactgggttc cggcggcaac tacagcgtca actggaacct gggttccggg aacatggtct gcggcaaagg ctggagtacc ggatcttcaa gccgcagaat cggctacaac gccggcgtct gggcgcgaa cggcaatgcc tacctgacct tgtatgggtg gaccaggaac tcgcctatcg agtactacgt ggtgacagt
                                                                                      180
                                                                                      240
                                                                                      300
                                                                                      360
                                                                                      420
tggggaaget ggaggeegee aggeggaace teegegggea eegteaatag egatggeggg
acctacaacc tctatcggac gcagcgggtc aacgcgcctt ccatcgacgg cacccggacg
ttctatcagt actggagtgt ccggacctcg aagaggccca ccgggagcaa ccagaccatc
                                                                                     480
                                                                                     540
accttcgcga accacgtgaa tgcgtggagg agcaaagggt ggaatctggg gagtcacgtc
                                                                                     600
taccagataa tggcaacaga gggatatcaa agcagcggga attccaacct gacggtgtgg
                                                                                     660
gcgcagtag
                                                                                     669
<210> 168
<211> 222
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(36)
<400> 168
Leu Phe Ser Ala Thr Gln Gln Leu Thr Ala Gln Thr Ile Cys Ser Asn 20 25 30
Gln Thr Gly Thr Asn Asn Gly Tyr Phe Tyr Ser Phe Trp Lys Asp Thr
35 40 45
Gly Ser Ala Cys Met Thr Leu Gly Ser Gly Gly Asn Tyr Ser Val Asn 50 60
Trp Asn Leu Gly Ser Gly Asn Met Val Cys Gly Lys Gly Trp Ser Thr 65 70 75 80
Gly Ser Ser Ser Arg Arg Ile Gly Tyr Asn Ala Gly Val Trp Ala Pro
85 90 95
Asn Gly Asn Ala Tyr Leu Thr Leu Tyr Gly Trp Thr Arg Asn Pro Leu 100 105 110
Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly Ser Trp Arg Pro Pro Gly
115 120 125
Gly Thr Ser Ala Gly Thr Val Asn Ser Asp Gly Gly Thr Tyr Asn Leu
130 140
Tyr Arg Thr Gln Arg Val Asn Ala Pro Ser Ile Asp Gly Thr Arg Thr
145 150 160
Phe Tyr Gln Tyr Trp Ser Val Arg Thr Ser Lys Arg Pro Thr Gly Ser
165 170 175
Asn Gln Thr Ile Thr Phe Ala Asn His Val Asn Ala Trp Arg Ser Lys
180
185
190
Gly Trp Asn Leu Gly Ser His Val Tyr Gln Ile Met Ala Thr Glu Gly
                                  200
Tyr Gln Ser Ser Gly Asn Ser Asn Leu Thr Val Trp Ala Gln 210 220
<210> 169
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<211> 1041

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<212> DNA
<213> Unknown
<223> Obtained from an environmental sample
<400> 169
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gccgcgcagg cgcaaacctg catcacttcc agccagaccg gtaccaacaa cggcaactac ttttccttct ggaaggacag cccgggtacc gtcaacttct gcatgtatgc caatgggcgc tacaacctcca actggagcgg catcaacaac tgggtgggcg gcaagggctg gcagacgggc tccaaccgca cggtgaccta ctccggttcg ttcaattcgc ccggcaatgg ctatctcacc ttgtacggat ggaccacgaa tccattgatc gagtactaca tcgtcgacag ctgggggcactattacgaccgc cgggcggcaa gggcttcatg ggcaccgtca acagcgatgg cggcacctat
                                                                                                      120
                                                                                                      180
                                                                                                      240
                                                                                                      300
                                                                                                      360
                                                                                                      420
gacatctacc gcacgcagcg cgtgaaccag ccttccatca tcggcaccgc cacgttctac
                                                                                                      480
cagtactgga gcgtgcggca gtcgaagcgc gtcggcggca cgatcaccac ggccaaccac ttcaacgcct gggccacgct gggcatgaac ctgggccagc acaactacca ggtcatggcc accgagggtt accagagcag tggcagctcc gacatcaccg tgaccgaggg cggcggctcc tcgtcgtcca gtggcggcgg cagcaccagc agtggcggtg gcggcagcaa gagcttcacc gtggcgtgcgc gcggcacggt cggcggcgaa aacatccagc tgcaggtcaa caaccagacg
                                                                                                      540
                                                                                                      600
                                                                                                      660
                                                                                                      720
                                                                                                      780
gtggcgagct ggaacctgac caccagcatg cagaactaca acgcctcgac cagcctgagt
                                                                                                      840
ggcggcatca ccgtcgtgta caccaatgac agcggcagcc gcgacgtgca ggtggactac atcgtcgtca acggccagac ccgccagtcc gaagcccaga gctacaacac cgggctctat
                                                                                                      900
                                                                                                     960
gccaacggac gtīgtggīgg cggctcgaac agcgagtgga igcattgcaa cggcgcgatt
                                                                                                     1020
ggctacggca acacgcccta g
                                                                                                    1041
<210> 170
<211> 346
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(24)
<400> 170
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                                                    10
Leu Gly Ile Thr Ala Ala Gln Ala Gln Thr Cys Ile Thr Ser Ser Gln
20 25 30
Thr Gly Thr Asn Asn Gly Asn Tyr Phe Ser Phe Trp Lys Asp Ser Pro
Gly Thr Val Asn Phe Cys Met Tyr Ala Asn Gly Arg Tyr Thr Ser Asn 50 _____ 60
Trp Ser Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Gln Thr Gly 65 70 75 80
Ser Asn Arg Thr Val Thr Tyr Ser Gly Ser Phe Asn Ser Pro Gly Asn 85 90 95
Gly Tyr Leu Thr Leu Tyr Gly Trp Thr Thr Asn Pro Leu Ile Glu Tyr
                                              105
Tyr Ile Val Asp Ser Trp Gly Thr Tyr Arg Pro Pro Gly Gly Gln Gly
115 120 125
Phe Met Gly Thr Val Asn Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Arg
130 140
Thr Gln Arg Val Asn Gln Pro Ser Ile Ile Gly Thr Ala Thr Phe Tyr 145 150 160
Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Val Gly Gly Thr Ile Thr
165 170 175
Thr Ala Asn His Phe Asn Ala Trp Ala Thr Leu Gly Met Asn Leu Gly
                                              185
Gln His Asn Tyr Gln Val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly
                                        200
Ser Ser Asp Ile Thr Val Thr Glu Gly Gly Ser Ser Ser Ser Ser 210 220
Gly Gly Gly Ser Thr Ser Ser Gly Gly Gly Ser Lys Ser Phe Thr
225 230 235 240
Val Arg Ala Arg Gly Thr Val Gly Gly Glu Asn Ile Gln Leu Gln Val
                                                     Page 133
```

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Asn Asn Gln Thr Val Ala Ser Trp Asn Leu Thr Thr Ser Met Gln Asn 260 265 270
Tyr Asn Ala Ser Thr Ser Leu Ser Gly Gly Ile Thr Val Val Tyr Thr
275 280 285
Asn Asp Ser Gly Ser Arg Asp Val Gln Val Asp Tyr Ile Val Val Asn 290 295 300
Gly Gln Thr Arg Gln Ser Glu Ala Gln Ser Tyr Asn Thr Gly Leu Tyr
305 310 320 320
Ala Asn Gly Arg Cys Gly Gly Gly Ser Asn Ser Glu Trp Met His Cys 325 330 335
Asn Gly Ala Ile Gly Tyr Gly Asn Thr Pro
340 345
<210> 171
<211> 678
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample
<400> 171
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                                                                                     60
                                                                                    120
ttcagtgtaa cgcagcagtc aaácgcccaa accatctgcá gcaatcaáac tggcacáaac
aacggtttct tctattcgtt ttggaaggac accggatcag catgcatgac tttgggctct ggcggcaatt acgacgtaag ttggaatctg ggttctggga atatggttgt cggcaaaggc tggagtaccg gatcatcaac caggaggagta ggctacaatg ccggcatctg gcagccgaac
                                                                                    180
                                                                                    240
                                                                                    300
ggcaatgcat atttggctct ctatgggtgg acgagaaacc cacttataga atattacgtc
                                                                                    360
gitgataget ggggcaettt caggccgcct ggaggaacgt caataggcic cgtcaccaet
                                                                                    420
gatggtggta cataccaaat atatcggacc cagcgagtca acgcgccttc cattgacggc
gccagaactt tttatcagta ctggagtgtc cggacctcga agagaccgac cgggagcaac
                                                                                    480
                                                                                    540
Caaaccatca cctttgcgaa tcacgttaac gcgtggagga atctaggttt gaatctgggg
                                                                                    600
agtcatgttt accagataat ggccacagag ggatttcata gcagtgggag atctaaccta
                                                                                    660
acggtgtggt cacagtaa
                                                                                    678
<210> 172
<211> 225
<212> PRT
<213> Unknown
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(29)
<400> 172
Met Glu Leu Lys Lys Ile Ser Arg Lys Gly Leu Pro Leu Val Phe Leu
1 5 10 15
Ser Leu Leu Phe Ser Val Thr Gln Gln Ser Asn Ala Gln Thr Ile
20 25 30
Cys Ser Asn Gln Thr Gly Thr Asn Asn Gly Phe Phe Tyr Ser Phe Trp
35 40 45
Lys Asp Thr Gly Ser Ala Cys Met Thr Leu Gly Ser Gly Gly Asn Tyr 50 60
Asp Val Ser Trp Asn Leu Gly Ser Gly Asn Met Val Val Gly Lys Gly 65 75 80
Trp Ser Thr Gly Ser Ser Thr Arg Arg Val Gly Tyr Asn Ala Gly Ile
Trp Gln Pro Asn Gly Asn Ala Tyr Leu Ala Leu Tyr Gly Trp Thr Arg
Asn Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly Thr Phe Arg
Pro Pro Gly Gly Thr Ser Ile Gly Ser Val Thr Thr Asp Gly Gly Thr 130 135 140
Tyr Gln Ile Tyr Arg Thr Gln Arg Val Asn Ala Pro Ser Ile Asp Gly
145 150 155 160
Ala Arg Thr Phe Tyr Gln Tyr Trp Ser Val Arg Thr Ser Lys Arg Pro
```

```
Thr Gly Ser Asn Gln Thr Ile Thr Phe Ala Asn His Val Asn Ala Trp
180

Arg Asn Leu Gly Leu Asn Leu Gly Ser His Val Tyr Gln Ile Met Ala
200
205
  Thr Glu Gly Phe His Ser Ser Gly Arg Ser Asn Leu Thr Val Trp Ser
  Gln
  225
 <210> 173
<211> 1503
<212> DNA
  <213> Unknown
  <220>
  <223> Obtained from an environmental sample
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                                                                                                                                                                                                       60
                                                                                                                                                                                                    120
                                                                                                                                                                                                     180
                                                                                                                                                                                                    240
                                                                                                                                                                                                    300
 gctggcaaaa ccgctagtgc ttacggcaat ataagcatta acttcgccgc tacgtggtct
tccggtgacg atgtgaagat gcttggcgta tatggttggg cgttttacgc actgccaagt
gtaccagaca aacaggaaaa cggcacttct actaattttt ccaatcaaat agaatactac
                                                                                                                                                                                                    360
                                                                                                                                                                                                    420
                                                                                                                                                                                                    480
 atcattcaag accgcggcag ctataactcg gctacaggtg gcaccaactc aaagaaatac ggtgaggcta ccattgacgg cattgcttat gagttccgtg tatgtgatag aatagggcaa cctatgttaa ctggcaacgg gaattttaag cagtatttca gtgttcctaa aagcactata aaccaccgca ccagcggtac aatcctgtt tccaaacact ttgaagaatg ggaaaaagtc ggcatgaaaa tggacggtcc cttatagta gtagcggtag aagttgaatc ctattctggc
                                                                                                                                                                                                    540
                                                                                                                                                                                                    600
                                                                                                                                                                                                    660
                                                                                                                                                                                                    720
                                                                                                                                                                                                    780
 aatgggaata gtaacggcaa tgctaaaatt acaaagaata ttitgaccat tggcggaaca
                                                                                                                                                                                                    840
 actygyddid gladcygcad tycladdil acddgyddid cillydcal lygcygadd
accacaactc aaagcagttc aagcggaggt tcaacggttc cagatgaatg tggcgaatat
aaaaagagtt tctgtggtgg cttgggatat ggaagcgtat attccaattt aaccgcaata
ccctcaacgg gcgactgctt atacatcgga gattttgaag taatccagcc agctttgaat
tcaaccgttg ccataaacgg tgtggaaaat acctgcggaa gcgagtggtc agattgccct
tacaatgata aacccgattc aaaaaaagat ggcggcatta atgtttatgt gaaaaacaggc
                                                                                                                                                                                                    900
                                                                                                                                                                                                   960
                                                                                                                                                                                                 1020
                                                                                                                                                                                                 1080
                                                                                                                                                                                                 1140
 tcaattaaca attatgagaa taacggttgg caaaacattg tagctaaagc aaaaccggct tgcacaccac cttctagcag ttccggtgct gcaccaggtt cttcttcttc agacgaagaa gacccagagc caatttgaa aaatcgcatt cctataactc atttttccct tcaaacgctt
                                                                                                                                                                                                 1200
                                                                                                                                                                                                 1260
                                                                                                                                                                                                 1320
agcgataaag ccttgcgcat agaagtaaat gctccaacta ttgtggacat ttttgacctg agagggaata aggttaaaag tttgaatgtt tacggttcgc aaagggttaa attatccctg ccgagcgggg tgtattttgc caaagtgcgc gggatgaaaa gcgttagatt tgtgttgagg
                                                                                                                                                                                                 1380
                                                                                                                                                                                                 1440
                                                                                                                                                                                                 1500
                                                                                                                                                                                                 1503
 <210> 174
 <211> 500
 <212> PRT
  <213> Unknown
 <223> Obtained from an environmental sample
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Leu Lys Lys Leu Ala Ala Ala Leu Ser Leu Ala Ile Thr Phe Ala Val 1 5 10 15

Pro Thr Ile Val Gln Ala Gln Gly Pro Thr Trp Thr Thr Ser Thr Ile 20 25 30

Gln Lys Tyr Asn Asn Tyr Asp Tyr Glu Leu Trp Asn Glu Asn Asn Gln 45

Gly Thr Val Ser Met Lys Leu Thr Gly Asp Asn Gly Thr Ala Ala Asn 50 60

Ala Val Gly Gly Thr Phe Glu Ser Thr Trp Ser Gly Thr Lys Asn Val 65 70 80

Leu Phe Arg Ser Gly Arg Lys Phe Thr Gly Thr Ser Gly Gln Ser Val
 Leu Phe Arg Ser Gly Arg Lys Phe Thr Gly Thr Ser Gly Gln Ser Val
```

```
Asp Gly Gly Ala Gly Lys Thr Ala Ser Ala Tyr Gly Asn Ile Ser
 Ile Asn Phe Ala Ala Thr Trp Ser Ser Gly Asp Asp Val Lys Met Leu
115 120 125
 Gly Val Tyr Gly Trp Ala Phe Tyr Ala Leu Pro Ser Val Pro Asp Lys
130
140
 Gln Glu Asn Gly Thr Ser Thr Asn Phe Ser Asn Gln Ile Glu Tyr Tyr
145 150 155 160
 Ile Ile Gln Asp Arg Gly Ser Tyr Asn Ser Ala Thr Gly Gly Thr Asn
165 170 175
 Ser Lys Lys Tyr Gly Glu Ala Thr Ile Asp Gly Ile Ala Tyr Glu Phe
180
180
190
Arg Val Cys Asp Arg Ile Gly Gln Pro Met Leu Thr Gly Asn Gly Asn 195

Phe Lys Gln Tyr Phe Ser Val Pro Lys Ser Thr Ile Asn His Arg Thr 210

Ser Gly Thr Ile Ser Val Ser Lys His Phe Glu Glu Trp Glu Lys Val 225

Gly Met Lys Met Asp Gly Pro Leu Tyr Glu Val Ala Met Lys Val Glu 245

Ser Tyr Ser Gly Asn Gly Asn Ser Asn Gly Asn Ala Lys Ile Thr Lys
 Ser Tyr Ser Gly Asn Gly Asn Ser Asn Gly Asn Ala Lys Ile Thr Lys 260 265 270
 Asn Ile Leu Thr Ile Gly Gly Thr Thr Thr Thr Gln Ser Ser Ser Ser 275 280 285
 Gly Gly Ser Thr Val Pro Asp Glu Cys Gly Glu Tyr Lys Lys Ser Phe
 Cys Gly Gly Leu Gly Tyr Gly Ser Val Tyr Ser Asn Leu Thr Ala Ile
305 310 315 320
 Pro Ser Thr Gly Asp Cys Leu Tyr Ile Gly Asp Phe Glu Val Ile Gln 325
 Pro Ala Leu Asn Ser Thr Val Ala Ile Asn Gly Val Glu Asn Thr Cys
340
350
350
350
350
350
350
350
Cys Thr Pro Pro Ser Ser Ser Ser Gly Ala Ala Pro Gly Ser Ser Ser 405 410 415
 Ser Asp Glu Glu Asp Pro Glu Pro Ile Leu Lys Asn Arg Ile Pro Ile
420 425 430
 Thr His Phe Ser Leu Gln Thr Leu Ser Asp Lys Ala Leu Arg Ile Glu
435 440 445
 Val Asn Ala Pro Thr Ile Val Asp Ile Phe Asp Leu Arg Gly Asn Lys
450 455 460
Val Lys Ser Leu Asn Val Tyr Gly Ser Gln Arg Val Lys Leu Ser Leu 465 470 475 480
Pro Ser Gly Val Tyr Phe Ala Lys Val Arg Gly Met Lys Ser Val Arg 485 490 495
 Phe Val Leu Arg
 <210> 175
 <211> 1053
 <212> DNA
 <213> Unknown
· <220>
 <223> Obtained from an environmental sample
 <400> 175
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                                                                                                        60
                                                                                                       120
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tactactaca cgttctggaa ggactcgggc agcgcctcga tgaccctcca tccgggggga cgctacagct cccagtggac cagcaacacc aacaactggg tcggcgggaa aggctggaat cccggtggc cgcggtggt caactactcg ggctactacg gggtcaacaa cagccagaac tcctacctgg cgctgtacgg ctggacccgc aatcgctgg tcgagtacta cgtgatcgag
                                                                                                       180
                                                                                                       240
                                                                                                       300
                                                                                                       360
```

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agctacggct cctacaaccc ggccagttgc gccggcgggg tggactacgg cagcttccag agcgatggcg ccacctacaa cgtacgtcgc tgcctgcgcc agaacgcgcc gtcgatcgaa
                                                                                    420
                                                                                    480
ggcaacaaca gcaccttcta ccagtacttc agcgtgcgca atcccaagaa gggattcggc
                                                                                    540
aacateteeg geaegateae egtegeeaae caetteaaet aetgggeeag eegeggeete
                                                                                    600
aacctcggca accacgacta catggtgttc gccaccgagg gctaccagag ccagggcagc
                                                                                    660
agcgacatca ccgtgagttc gggtaccggc ggcggcggtg gcggcggcaa cacgggcagc aagaccatcg tggtgcgcg gcgcggcacc gccggcggag agaacatctc gctcaaggtcaacaacgcca ccatcgccag ctggacgctc accaccagca tggccaacta cacggccacc
                                                                                    720
                                                                                    780
                                                                                    840
acctcggcat cgggcggctc gctggtggag ttcaccaacg acggcggcaa ccgcgacgtg
                                                                                    900
caggtggact acctcagcgt caatggcgcc gtccgccagg ccgaggacca gacctacaac
                                                                                    960
1020
                                                                                   1053
<210> 176
<211> 350
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(27)
<400> 176
Met Lys Ser Ile Arg Ser Arg Ser Leu Ala Thr Ala Val Leu Ala Gly
1 5 10 15
Ala Leu Gly Val Ala Ala Ala Gly Ala Gln Ala Gln Thr Leu Asn Asn 20 25 30
Asn Ser Thr Gly Thr His Asp Gly Tyr Tyr Tyr Thr Phe Trp Lys Asp 35 40 45
Ser Gly Ser Ala Ser Met Thr Leu His Pro Gly Gly Arg Tyr Ser Ser
50 60
Gln Trp Thr Ser Asn Thr Asn Asn Trp Val Gly Gly Lys Gly Trp Asn 65 75 80
Pro Gly Gly Pro Arg Val Val Asn Tyr Ser Gly Tyr Tyr Gly Val Asn 90. 95
Asn Ser Gln Asn Ser Tyr Leu Ala Leu Tyr Gly Trp Thr Arg Asn Pro 100 	 105 	 110
Leu Val Glu Tyr Tyr Val Ile Glu Ser Tyr Gly Ser Tyr Asn Pro Ala
115 120 125
Ser Cys Ala Gly Gly Val Asp Tyr Gly Ser Phe Gln Ser Asp Gly Ala
130 135 140
Thr Tyr Asn Val Arg Arg Cys Leu Arg Gln Asn Ala Pro Ser Ile Glu
145 150 160
Gly Asn Asn Ser Thr Phe Tyr Gln Tyr Phe Ser Val Arg Asn Pro Lys
165 170 175
Lys Gly Phe Gly Asn Ile Ser Gly Thr Ile Thr Val Ala Asn His Phe
180 185
Asn Tyr Trp Ala Ser Arg Gly Leu Asn Leu Gly Asn His Asp Tyr Met
195 200 205
Val Phe Ala Thr Glu Gly Tyr Gln Ser Gln Gly Ser Ser Asp Ile Thr
210 215 220
Val Ser Ser Gly Thr Gly Gly Gly Gly Gly Gly Asn Thr Gly Ser
225 230 235 240
Lys Thr Ile Val Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Asn Ile
245 250 255
Ser Leu Lys Val Asn Asn Ala Thr Ile Ala Ser Trp Thr Leu Thr Thr 260 265 270
Ser Met Ala Asn Tyr Thr Ala Thr Thr Ser Ala Ser Gly Gly Ser Leu
275 280 285
Val Glu Phe Thr Asn Asp Gly Gly Asn Arg Asp Val Gln Val Asp Tyr
290 _____ 295 ____ 300 ____
Leu Ser Val Asn Gly Ala Val Arg Gln Ala Glu Asp Gln Thr Tyr Asn 305 310 315 320
Thr Gly Val Tyr Gln Asn Gly Gln Cys Gly Gly Gly Asn Gly Arg Ser
Glu Trp Leu His Cys Asn Gly Ala Ile Gly Phe Gly Asn 340 345
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<210> 177
 <211> 1299
 <212> DNA
 <213> Unknown
 <220>
 <223> Obtained from an environmental sample
 <400> 177
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                                                                                                                                      60
                                                                                                                                    120
                                                                                                                                    180
                                                                                                                                    240
                                                                                                                                    300
                                                                                                                                    360
                                                                                                                                    420
                                                                                                                                    480
 taccaatact ggagtgtgcg ccaaaacaag cgcaccagcg gaacgattaa tattggagcg
                                                                                                                                    540
 catttcgatg catgggctgc tgtgggcttg aacctgggga ctcacgatta tcagattatg
                                                                                                                                    600
 gcgaccgagg gctaccagag cagcggccag tccaatatca cggtgagcga aggcagtagcggcagcacga cttcgagcac atccagctcc agctcaagta cgagttccag tagttcttcc
                                                                                                                                    660
                                                                                                                                    720
 agcagttctt ccggcggcgg cacaggaagt tgtgccggag tgaatgtgta ccccaattgg accgcacgcg actggtctgg cggcgcatac aatcacgcca atgccggtga ccaaatggtc
                                                                                                                                    780
                                                                                                                                    840
tatcaaaaca atttgtaccg ggcaaactgg tacaccaact ccacgcctgg aagcgatgcc tcctggacca gtctcgggtc ctgtagcggc ggcggtagca ccagttcaac aacgagctcc tccagttcct cttccacctc ggcgtcgagc agctccaact catccagcag cagttcaagc
                                                                                                                                    900
                                                                                                                                    960
                                                                                                                                  1020
 agctccagca gcggtggctg tcgggaaatg tgtaactggt acggacaggg tatgtatcct
                                                                                                                                  1080
ctgtgtcaga acaccagcgg ttggggatgg gaaaataacc agaactgtat cggtcgccaa acctgtcaaa gtcagaacgg cggctccggg ggtgtggtga acagctgtgg taccagcagc tcttcgtcca gtagcacctc ctcatcgagc agttcaagtt cgtcgagtgg caccacgtca
                                                                                                                                  1140
                                                                                                                                  1200
                                                                                                                                  1260
 tcgtcctccg gaattcctgc agcccggggg atccactag
                                                                                                                                  1299
 <210> 178
<211> 432
 <212> PRT
 <213> Unknown
 <220>
 <223> Obtained from an environmental sample
 <221> SIGNAL
 <222> (1)...(26)
Met Lys Leu Leu Lys Thr His Arg Arg Ala Ile Ala Ala Ala Ala Leu 1 10 15 Ala Val Ala Thr Val Pro Ile Ala His Ala Gln Thr Leu Ser Ser Asn 20 25 30
Ala Thr Gly Thr Gln Asn Gly Tyr Tyr Tyr Ser Phe Trp Lys Asp Ser
Gly Asn Ala Thr Met Thr Leu Gly Ala Gly Gly Asn Tyr Ser Ser Ser 50 55 60
Trp Asn Ser Ser Thr Asn Asn Trp Val Gly Gly Lys Gly Trp Met Pro
Gly Thr Arg Arg Thr Val Thr Tyr Ser Gly Ser Tyr Ser Ala Ser Gly
Thr Ser Tyr Leu Ala Leu Tyr Gly Trp Thr Arg Asn Pro Leu Île Glu
100 110
Tyr Tyr Ile Val Glu Asn Trp Val Asn Tyr Asn Pro Ala Ser Gly Ala
115 120 125
Thr Asn Tyr Gly Thr Val Asn Tle Asp Gly Ser Thr Tyr Gln Leu Gly
Arg Ser Gln Arg Val Asn Gln Pro Ser Ile Glu Gly Thr Ala Thr Phe 145 150 150 160

Tyr Gln Tyr Trp Ser Val Arg Gln Asn Lys Arg Thr Ser Gly Thr Ile 165 170 175

Asn Ile Gly Ala His Phe Asp Ala Trp Ala Ala Val Gly Leu Asn Leu 180 185
```

```
Gly Thr His Asp Tyr Gln Ile Met Ala Thr Glu Gly Tyr Gln Ser Ser
195 200 205
                                    200
Gly Gln Ser Asn Ile Thr Val Ser Glu Gly Ser Ser Gly Ser Thr Thr
210 215 220
225
                          230
                                                    235
Ser Ser Ser Ser Gly Gly Gly Thr Gly Ser Cys Ala Gly Val Asn Val 245 250 255
Tyr Pro Asn Trp Thr Ala Arg Asp Trp Ser Gly Gly Ala Tyr Asn His
260 265 270
Ala Asn Ala Gly Asp Gln Met Val Tyr Gln Asn Asn Leu Tyr Arg Ala
275 280 285
Asn Trp Tyr Thr Asn Ser Thr Pro Gly Ser Asp Ala Ser Trp Thr Ser 290 295 300
Leu Gly Ser Cys Ser Gly Gly Gly Ser Thr Ser Ser Thr Thr Ser Ser 305 310 315
                                                    315
                                                                             320
Ser Ser Ser Ser Ser Ser Ser Gly Gly Cys Arg Glu Met Cys Asn 340 345 _ 350 _
Trp Tyr Gly Gln Gly Met Tyr Pro Leu Cys Gln Asn Thr Ser Gly Trp 355 360 365
Gly Trp Glu Asn Asn Gln Asn Cys Ile Gly Arg Gln Thr Cys Gln Ser
370 375 380
Gln Asn Gly Gly Ser Gly Gly Val Val Asn Ser Cys Gly Thr Ser Ser 385 395 400
405
                                              410
Gly Thr Thr Ser Ser Ser Ser Gly Ile Pro Ala Ala Arg Gly Ile His
420
430
<210> 179
<211> 852
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 179
atgaagaatt ggccgggaac gggtattata ttattattgg cgggcggcct tttggcggct
                                                                                           60
tgtttgacgg gcaaacggca agaggggcaa aaagtggatc cggatactca aaacgagaaa ttgacaggcg ggaccgtgtt tacagctaac agcaggggga acaggcccct ggaaggttcg ccttatggtt acgaaatggt gacgcagggc gggaataata acaagcttgt ttggttcggg ccggatcagg ggggaatgga aggagcgga tgatttttg
                                                                                          120
                                                                                          180
                                                                                          240
                                                                                          300
ggacgactgg gtttctggtg gggaaacggc gggcaattta aagaatataa aaatatgtac
                                                                                          360
gcggatttca attacacaag gtcggggcgc ggcaccggcg gcagttattc ttatataggc atttacggct gggcgagaaa cccgaacgcc gcgaacgagg aagacaggtt aatagaatac tatattgtgg acgactggtt cgggaatcaa tggcagtccg acgacacccc cattaccaca
                                                                                          420
                                                                                          480
                                                                                          540
agaacaacag gaggctccgt attgggtacc attatagcgg acggcgcgtt ttacaacgtc gtcaggaatg tgagaaccca aaagccttcg atagacggca tcaaaacatt cgcccaatac ttcagcatac gccaaacacc gcgccaaagc gggacaatct ccatcaccga acatttcaaa caatgggaaa gcatgggct gaagctcgga aatagtacg aggacaaaatt cctggtagaa
                                                                                          600
                                                                                          660
                                                                                          720
                                                                                          780
gccggcggcg gcaccggctg gctggagttt acgtatctta aactgacgca ggaagaaaaa
                                                                                          840
aaaagaaatt ag
                                                                                          852
<210> 180
<211> 283
<212> PRT
<213> Unknown
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(19)
<400> 180
Met Lys Asn Trp Pro Gly Thr Gly Ile Ile Leu Leu Leu Ala Gly Gly
```

```
Leu Leu Ala Ala Cys Leu Thr Gly Lys Arg Gln Glu Gly Gln Lys Val
 Asp Pro Asp Thr Gln Asn Glu Lys Leu Thr Gly Gly Thr Val Phe Thr
35 40 45
 Ala Asn Ser Arg Gly Asn Arg Pro Leu Glu Gly Ser Pro Tyr Gly Tyr
50 55 60
Glu Met Trp Thr Gln Gly Gly Asn Asn Asn Lys Leu Val Trp Phe Gly 65 75 80
Pro Asp Gln Gly Gly Ala Ala Phe Arg Ala Glu Trp Asn Glu Pro 85 90 95
Asp Asp Phe Leu Gly Arg Leu Gly Phe Trp Trp Gly Asn Gly Gly Gln 100 105 110
 Phe Lys Glu Tyr Lys Asn Met Tyr Ala Asp Phe Asn Tyr Thr Arg Ser
Gly Arg Gly Thr Gly Gly Ser Tyr Ser Tyr Ile Gly Ile Tyr Gly Trp
Ala Arg Asn Pro Asn Ala Ala Asn Glu Glu Asp Arg Leu Ile Glu Tyr
145 _____ 150 ____ 160
Tyr Ile Val Asp Asp Trp Phe Gly Asn Gln Trp Gln Ser Asp Asp Thr
165 170 175
Pro Ile Thr Thr Arg Thr Thr Gly Gly Ser Val Leu Gly Thr Ile Ile
180 185 190
Ala Asp Gly Ala Phe Tyr Asn Val Val Arg Asn Val Arg Thr Gln Lys
200
205
Pro Ser Ile Asp Gly Ile Lys Thr Phe Ala Gln Tyr Phe Ser Ile Arg
210 215 220
Gln Thr Pro Arg Gln Ser Gly Thr Ile Ser Ile Thr Glu His Phe Lys 235 230 240
Gln Trp Glu Ser Met Gly Leu Lys Leu Gly Asn Met Tyr Glu Ala Lys 245 250 255
Phe Leu Val Glu Ala Gly Gly Gly Thr Gly Trp Leu Glu Phe Thr Tyr 260 265 270
Leu Lys Leu Thr Gln Glu Glu Lys Lys Arg Asn
275 280
<210> 181
<211> 1077
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 181
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                                                                                           60
                                                                                          120
                                                                                          180
                                                                                          240
gggcgacgca ccgtcaccta ttccggcacc tacaacccca atggcaattc gtacctgacc
                                                                                          300
ctgtacggct ggaccacgaa tccactggtc gagtactaca tcgtcgacag ctggggttcc
tggcgcccac cgggctcggg atacatgggc acggtcacca gcgatggcgg cacctacgac
atctatcgca cgcagcgtg gaaccagcct tccatcatcg gcaccgcgac gttctaccaa
                                                                                          360
                                                                                          420
480
                                                                                          540
                                                                                          600
                                                                                          660
                                                                                          720
                                                                                          780
                                                                                          840
                                                                                          900
                                                                                          960
                                                                                         1020
tctaacagcg agtggatgca ctgcaacggc gccatcggct acggcaacac gccctag
                                                                                         1077
<210> 182
<211> 358
<212> PRT
<213> Unknown
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<220>
 <223> Obtained from an environmental sample
 <221> SIGNAL
 <222> (1)...(25)
 <400> 182
 Met Asn Phe Ser Leu Arg Lys Ala Ala Ala Ala Leu Ala Cys Val Ala
10 15
 Gly Leu Tyr Ala Ser Ser Ala Gly Ala Gln Thr Cys Leu Thr Asn Asn 20 25 30
 Gln Thr Gly Asn Asn Gly Gly Tyr Tyr Tyr Ser Phe Trp Lys Asp Ser
 Gly Asn Val Thr Phe Cys Leu Gln Ser Gly Gly Arg Tyr Thr Ser Gln 50 60
 Trp Ser Asn Val Asn Asn Trp Val Gly Gly Lys Gly Trp Asn Pro Gly 65 70 75 80
 Gly Arg Arg Thr Val Thr Tyr Ser Gly Thr Tyr Asn Pro Asn Gly Asn 90 95
 Ser Tyr Leu Thr Leu Tyr Gly Trp Thr Thr Asn Pro Leu Val Glu Tyr
100 105 110
Tyr Ile Val Asp Ser Trp Gly Ser Trp Arg Pro Pro Gly Ser Gly Tyr
115 120 125
Met Gly Thr Val Thr Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Arg Thr
Gln Arg Val Asn Gln Pro Ser Ile Ile Gly Thr Ala Thr Phe Tyr Gln
145 150 155 160
Tyr Trp Ser Val Arg Gln Ser Lys Arg Val Gly Gly Thr Ile Thr Ser
Gly Asn His Phe Asp Ala Trp Ala Ser Leu Gly Met Asn Leu Gly Thr
180 ______ 185 _____ 190
His Asn Tyr Met Val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser
195 200 205
Ser Ser Ser Gly Gly Gly Gly Thr Lys Ser Phe Thr Val Arg Ala Arg 255
Gly Thr Ala Gly Gly Glu Ser Ile Thr Leu Arg Val Asn Asn Gln Asn 260 265 270
Val Gln Thr Trp Thr Leu Gly Thr Ser Met Gln Asn Tyr Thr Ala Ser
275 280 285
Thr Ser Leu Ser Gly Gly Ile Thr Val Ala Phe Thr Asn Asp Gly Gly
                            295
                                                   300
Asn Arg Asp Val Gln Val Asp Tyr Ile Ile Val Asn Gly Gln Thr Arg 305 310 315 320
Gln Ser Glu Ala Gln Thr Tyr Asn Thr Gly Leu Tyr Ala Asn Gly Arg
Cys Gly Gly Ser Asn Ser Glu Trp Met His Cys Asn Gly Ala Ile
340 345 350
Gly Tyr Gly Asn Thr Pro
355
<210> 183
<211> 1083
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample
<400> 183
atgatcgaag gtctcaggag acctgccttc agtggcagga gcatcgtcaa ggcattgctc
tgcgtcgcgg ccctgtatgc atcggcggcg caggcgcaga cctgtctcag ttcgagccag
accggcacca acaacggctt ctactattcg ttctggaagg acagcccggg cagcgtgcag
                                                                                  60
                                                                                 120
                                                                                 180
ttctgcatgt attccggcgg ccgctacaca tccaactgga gcggcatcaa caactgggtc
ggcggcaagg ggtggcagac cggcgcctcg cgcgtggtca gctactcggg cacgttcaat
                                                                                 240
                                                                                 300
tčaččgggča acggetačet ggčgčtgtač ggctggacca ccaatccact ggtcgagtac
                                                                                 360
                                          Page 141
```

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tacatcgtcg acaactgggg cacctatcgc ccgccgggcg gcacgggatt ccagggcacg
                                                                                    420
gtgaccagtg acggcggtac ctacgacatc taccggaccg agcgcaccaa cgcgccctgc
                                                                                    480
atcaccggca acaactgcaa cttctcgcag ttctggagcg tgcggcagtc gaagcgcacc
ggcggcacca tcaccaccgg caatcacttc agcgcctggg cgtcgcacgg catgaacatg
                                                                                    540
                                                                                    600
ggccagcaca actaccagat catggccacc gagggttacc agagcaacgg cagctcggac
                                                                                    660
atcacggtct cggaaggcag cagttcgtcg agcagcagca gttcgtcctc ttcgtcgagc
                                                                                    720
agcagetegt egageggegg eggeggeage aagagettea eggtgegege eegeggeace
                                                                                    780
gcgggtggcg agcagatccg gctgcgcgtg aacaatacga ccgtgcagac ctggacgctg
aacaccacga tgacgaacta caccgcttcg accacgctga gcggcggcat cacggtggag
                                                                                    840
                                                                                    900
tacttcaacg acagcaccaa tcacgacgtg caggtggact acatcatcgt gaacggcgcg acgcgccagt ccgaagcgca gagctacaac accggcctgt atgccaacgg ccgttgcggt
                                                                                    960
                                                                                   1020
ggcggttcca acagcgaatg gatgcattgc aatggcgcca tcggctacgg caacactcca
                                                                                   1080
                                                                                   1083
<210> 184
<211> 360
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(32)
<400> 184
Met Ile Glu Gly Leu Arg Arg Pro Ala Phe Ser Gly Arg Ser Ile Val
1 5 10
Lys Ala Leu Cys Val Ala Ala Leu Tyr Ala Ser Ala Ala Gln Ala 20 25 30
Gln Thr Cys Leu Ser Ser Ser Gln Thr Gly Thr Asn Asn Gly Phe Tyr 35 40 45
Tyr Ser Phe Trp Lys Asp Ser Pro Gly Ser Val Gln Phe Cys Met Tyr 50 60
Ser Gly Gly Arg Tyr Thr Ser Asn Trp Ser Gly Ile Asn Asn Trp Val
65 70 75 80
Gly Gly Lys Gly Trp Gln Thr Gly Ala Ser Arg Val Val Ser Tyr Ser
85 90 95
Gly Thr Phe Asn Ser Pro Gly Asn Gly Tyr Leu Ala Leu Tyr Gly Trp
Thr Thr Asn Pro Leu Val Glu Tyr Tyr Ile Val Asp Asn Trp Gly Thr
Tyr Arg Pro Pro Gly Gly Thr Gly Phe Gln Gly Thr Val Thr Ser Asp
130 135 140
Gly Gly Thr Tyr Asp Ile Tyr Arg Thr Glu Arg Thr Asn Ala Pro Cys
145 155 160
lle Thr Gly Asn Asn Cys Asn Phe Ser Gln Phe Trp Ser Val Arg Gln
165 170 175
Ser Lys Arg Thr Gly Gly Thr Ile Thr Thr Gly Asn His Phe Ser Ala
180
185
190
187
Trp Ala Ser His Gly Met Asn Met Gly Gln His Asn Tyr Gln Ile Met
Ala Thr Glu Gly Tyr Gln Ser Asn Gly Ser Ser Asp Ile Thr Val Ser 210 215 220
Ser Ser Ser Ser Gly Gly Gly Gly Ser Lys Ser Phe Thr Val Arg
Ala Arg Gly Thr Ala Gly Gly Glu Gln Ile Arg Leu Arg Val Asn Asn 260 270
                                      265
Thr Thr Val Gln Thr Trp Thr Leu Asn Thr Thr Met Thr Asn Tyr Thr 275 280 285
Ala Ser Thr Thr Leu Ser Gly Gly Ile Thr Val Glu Tyr Phe Asn Asp 290 295 300
Ser Thr Asn His Asp Val Gln Val Asp Tyr Ile Ile Val Asn Gly Ala 305 310 315 320 Thr Arg Gln Ser Glu Ala Gln Ser Tyr Asn Thr Gly Leu Tyr Ala Asn 325 330 335 61
Gly Arg Cys Gly Gly Gly Ser Asn Ser Glu Trp Met His Cys Asn Gly
```

```
340
                                                                350
Ala Ile Gly Tyr Gly Asn Thr Pro
355 360
<210> 185
<211> 684
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 185
atgaatttga aaagattgag gctgttgttt gtgatgtgta ttggatttgt gctgacactg
                                                                                       60
acggctgtgc cagctcatgc ggaaacgatt tatgataata ggatagggac acacagcgga
                                                                                      120
tacgattitg aattatggaa ggattacgga aatacctcga tgacactcaa taacggcggg
                                                                                      180
gcatttagtg caagctggaa caatattgga aatgccttat ttcgaaaagg aaagaagttt gattccacta aaactcatca tcaacttggc aacatctcca tcaactacaa cgcagccttt
                                                                                      240
                                                                                      300
aacccgggcg ggaattccta tttatgtgtc tatggctgga cacaatctcc attagctgaa
                                                                                      360
tactacattg tigagtcatg gggcacatat cgtccaacag gaacgtataa aggatcattt tatgccgatg gaggcacata tgacatatat gaaacgctcc gtgtcaatca gccttctatc
                                                                                      420
                                                                                      480
attggagacg ctaccttcaa acaatattgg agtgtacgtc aaacaaaacg cacaagcgga actgtttccg tcagtgagca ttttaaaaaaa tgggaaagct taggcatgcc aatgggaaaa
                                                                                      540
                                                                                      600
atgiatgaaa cagcaitaac tgtagaaggc taccgaagca acggaagigc gaaigtcaig
                                                                                      660
acgaatcagc tgatgattcg ataa
                                                                                      684
<210> 186
<211> 227
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(27)
<400> 186
Met Asn Leu Lys Arg Leu Arg Leu Leu Phe Val Met Cys Ile Gly Phe
1 10 15
Val Leu Thr Leu Thr Ala Val Pro Ala His Ala Glu Thr Ile Tyr Asp
20 25 30
Asn Arg Ile Gly Thr His Ser Gly Tyr Asp Phe Glu Leu Trp Lys Asp 45
Tyr Gly Asn Thr Ser Met Thr Leu Asn Asn Gly Gly Ala Phe Ser Ala 50 60
Ser Trp Asn Asn Ile Gly Asn Ala Leu Phe Arg Lys Gly Lys Lys Phe 65 70 75 80
Asp Ser Thr Lys Thr His His Gln Leu Gly Asn Ile Ser Ile Asn Tyr
85 90 95
Asn Ala Ala Phe Asn Pro Gly Gly Asn Ser Tyr Leu Cys Val Tyr Gly
100 105 110
Trp Thr Gln Ser Pro Leu Ala Glu Tyr Tyr Ile Val Glu Ser Trp Gly
115 120 125
Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Ser Phe Tyr Ala Asp Gly 130 135 140
Gly Thr Tyr Asp Ile Tyr Glu Thr Leu Arg Val Asn Gln Pro Ser Ile
145 150 160
Ile Gly Asp Ala Thr Phe Lys Gln Tyr Trp Ser Val Arg Gln Thr Lys
165 170 175
Arg Thr Ser Gly Thr Val Ser Val Ser Glu His Phe Lys Lys Trp Glu
180 185 190
Ser Leu Gly Met Pro Met Gly Lys Met Tyr Glu Thr Ala Leu Thr Val
195 200 _ 205
Glu Gly Tyr Arg Ser Asn Gly Ser Ala Asn Val Met Thr Asn Gln Leu
210 215 220
Met Ile Arg
225
```

60

```
<210> 187
<211> 642
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 187
atgtttaagt ttaaaaagaa tttcttagtt ggattatcgg cagctttaat gagtattagc
ttgttttcgg caaccgcctc tgcagctagc acagactact ggcaaaattg gactgatggg
                                                                                    120
ggcggtatág taaacgctgt cáatgggtét ggcgggaatt ácagtgttaá ttggfetáát
                                                                                    180
accggaaatt ttgttgttgg taaaggttgg actacaggtt cgccatttag gacgataaac tataatgccg gagtttgggc gccgaatggc aatggatatt taactttata tggttggacg agatcacctc tcatagaata ttatgtagtg gattcatggg gtacttatag acctactgga
                                                                                    240
                                                                                    300
                                                                                    360
                                                                                    420
acgtataaag gtactgtaaa aagtgatggg ggtacatatg acatatatac aactacacgt
                                                                                    480
tataacgcac cttccattga tggcgatcgc actactttta cgcagtactg gagtgttcgc
cagtcgaaga gaccaaccgg aagcaacgct acaatcactt tcagcaatca tgtgaacgca tggaagagcc atggaatgaa tctgggcagt aattgggctt accaagtcat ggcgacagaa
                                                                                    540
                                                                                    600
ggatatcaaa gtagtggaag ttctaacgta acagtgtggt aa
                                                                                    642
<210> 188
<211> 213
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(28)
<400> 188
Met Phe Lys Phe Lys Lys Asn Phe Leu Val Gly Leu Ser Ala Ala Leu 1 5 10 15
Met Ser Ile Ser Leu Phe Ser Ala Thr Ala Ser Ala Ala Ser Thr Asp 20 25 30 _
Tyr Trp Gln Asn Trp Thr Asp Gly Gly Gly Ile Val Asn Ala Val Asn 35 40 45
Gly Ser Gly Gly Asn Tyr Ser Val Asn Trp Ser Asn Thr Gly Asn Phe 50 60
Val Val Gly Lys Gly Trp Thr Thr Gly Ser Pro Phe Arg Thr Ile Asn 65 70 75 80
Tyr Asn Ala Gly Val Trp Ala Pro Asn Gly Asn Gly Tyr Leu Thr Leu
85 90 95
Tyr Gly Trp Thr Arg Ser Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser
Trp Gly Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Thr Val Lys Ser
Asp Gly Gly Thr Tyr Asp Ile Tyr Thr Thr Thr Arg Tyr Asn Ala Pro
130 140
Ser Ile Asp Gly Asp Arg Thr Thr Phe Thr Gln Tyr Trp Ser Val Arg
Gln Ser Lys Arg Pro Thr Gly Ser Asn Ala Thr Ile Thr Phe Ser Asn 165 170 175
His Val Asn Ala Trp Lys Ser His Gly Met Asn Leu Gly Ser Asn Trp
180 185 190
Ala Tyr Gln Val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser Ser
195 200 205
Asn Val Thr Val Trp
    210
<210> 189
<211> 570
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
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<400> 189
 atggccctta tggcttcgac agactactgg caaaattgga ctgatggtgg tgggacagta aatgctacca atggatctga tggcaattac agcgtttcat ggtcaaattg cgggaattt
                                                                                                           60
                                                                                                          120
 gttgttggta aaggctggac taccggatca gcaactaggg taataaacta taatgccgga
                                                                                                          180
gccttttcgc cgtccggtaa tggatatttg gctctttatg ggtggacgag aaattcactc atagaatatt acgtcgttga tagctggggg acttatagac ctactggaac ttataaaggc actgtggacta gtgatggagg gacttatgac atatacacga ctactggaac caacgcacct tccattgacg gcaataatac aactttcacc cagtctgga gtgtatggaag gcaataatac actttcacc cagtctcgaa gtgtataggaagaa
                                                                                                          240
                                                                                                          300
                                                                                                          360
                                                                                                          420
 ccgattggta ccaacaatac catcaccttt agcaaccatg ttaacgcctg gaagagtaaa
                                                                                                          480
 ggaatgaatt tggggagtag ttggtcttat caggtattag caacagaggg ctatcaaagt
                                                                                                          540
 agtgggtact ctaacgtaac ggtctggtaa
                                                                                                          570
 <210> 190
 <211> 189
<212> PRT
 <213> Unknown
 <220>
 <223> Obtained from an environmental sample
Met Ala Leu Met Ala Ser Thr Asp Tyr Trp Gln Asn Trp Thr Asp Gly \frac{1}{1} \frac{1}{1}
Gly Gly Thr Val Asn Ala Thr Asn Gly Ser Asp Gly Asn Tyr Ser Val
Ser Trp Ser Asn Cys Gly Asn Phe Val Val Gly Lys Gly Trp Thr Thr
Gly Ser Ala Thr Arg Val Ile Asn Tyr Asn Ala Gly Ala Phe Ser Pro
50 60
Ser Gly Asn Gly Tyr Leu Ala Leu Tyr Gly Trp Thr Arg Asn Ser Leu 65 70 75 80
Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly Thr Tyr Arg Pro Thr Gly
Thr Tyr Lys Gly Thr Val Thr Ser Asp Gly Gly Thr Tyr Asp Ile Tyr 100 105 110
Thr Thr Arg Thr Asn Ala Pro Ser Ile Asp Gly Asn Asn Thr Thr
Phe Thr Gln Phe Trp Ser Val Arg Gln Ser Lys Arg Pro Ile Gly Thr
Asn Asn Thr Ile Thr Phe Ser Asn His Val Asn Ala Trp Lys Ser Lys
145 150 160
Gly Met Asn Leu Gly Ser Ser Trp Ser Tyr Gln Val Leu Ala Thr Glu
165 170 175
Gly Tyr Gln Ser Ser Gly Tyr Ser Asn Val Thr Val Trp
<210> 191
<211> 1053
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 191
atgaagtcca ttcgcagccg cagcctcgcc accgccgtcc tggctggcgc cctcggcgtc
                                                                                                          60
gcagccgccg gcgcgcaggc gcagacgctc aacaacaatt ccaccggcac gcacgacggc
                                                                                                         120
ttctactaca cgttctggaa ggactcgggc agcgcctcga tgaccctcca tccgggcgga cgctacagct cccagtggac cagcaacacc aacaactggg tcggcgggaa aggctggaat cccggtggc cgcgctggt caactactcg ggctactacg gggtcaacaa cagccagaac tcctacctgg cgctgtacgg ctggaccgc aatccgctgg tggactacta cgtgatcgag agctacggct cctacaaccc ggccagttgc gccggcgggg tggactacgg cagcttccag agcgatggcg ccacctacaa cgtacgccgc tgcctgcgca agaacgcgcc gtcgatcgaa
                                                                                                         180
                                                                                                         240
                                                                                                         300
                                                                                                         360
                                                                                                         420
                                                                                                         480
ggcaacaaca gcaccttcta ccagtacttc agcgtgcgca atcccaagaa gggattcggcaacatctccg gcacgatcac cgtcgccaac cacttcaact actgggccag ccgcggcctc
                                                                                                         540
                                                                                                         600
aacctcggca accacgacta catggtgttc gccaccgagg gctaccagag ccagggcagc agcgacatca ccgtgagttc gggtaccggc ggcggcggtg gcggcggcaa cacgggcagc
                                                                                                         660
                                                                                                         720
aagaccatcg tggtgcgcgc gcgcggcacc gccggcggag agaacatctc gctcaaggtc
                                                                                                         780
                                                       Page 145
```

```
aacaacgcca ccatcgccag ctggacgctc accaccagca tggccaacta cacggccacc
                                                                              840
acctcggcat cgggcggctc gctggtggag ttcaccaacg acggcggcaa ccgcgacgtg
                                                                              900
caggtggact acctcagcgt caatggcgcc gtccgccagg ccgaggacca gacctacaac
                                                                              960
accggcgtgt accagaacgg ccagtgcggc ggcggcaacg gccgcagcga atggctgcac
                                                                             1020
tgcaacggtg ccatcggctt cggaaatctc tga
                                                                             1053
<210> 192
<211> 350
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(27)
<400> 192
Met Lys Ser Ile Arg Ser Arg Ser Leu Ala Thr Ala Val Leu Ala Gly
Ala Leu Gly Val Ala Ala Ala Gly Ala Gln Ala Gln Thr Leu Asn 20 25 30
Asn Ser Thr Gly Thr His Asp Gly Phe Tyr Tyr Thr Phe Trp Lys Asp
Ser Gly Ser Ala Ser Met Thr Leu His Pro Gly Gly Arg Tyr Ser Ser 50 60
Gln Trp Thr Ser Asn Thr Asn Asn Trp Val Gly Gly Lys Gly Trp Asn 65 _ _ _ 80
Pro Gly Gly Pro Arg Val Val Asn Tyr Ser Gly Tyr Tyr Gly Val Asn
85 90 _____ 95
Asn Ser Gln Asn Ser Tyr Leu Ala Leu Tyr Gly Trp Thr Arg Asn Pro
Leu Val Glu Tyr Tyr Val Ile Glu Ser Tyr Gly Ser Tyr Asn Pro Ala
Ser Cys Ala Gly Gly Val Asp Tyr Gly Ser Phe Gln Ser Asp Gly Ala
130 135 140
Thr Tyr Asn Val Arg Arg Cys Leu Arg Gln Asn Ala Pro Ser Ile Glu
145 150 160
Gly Asn Asn Ser Thr Phe Tyr Gln Tyr Phe Ser Val Arg Asn Pro Lys
165 170 175
Lys Gly Phe Gly Asn Ile Ser Gly Thr Ile Thr Val Ala Asn His Phe 180 185 190
Asn Tyr Trp Ala Ser Arg Gly Leu Asn Leu Gly Asn His Asp Tyr Met 200 205
Val Phe Ala Thr Glu Gly Tyr Gln Ser Gln Gly Ser Ser Asp Ile Thr 210 215 220
Val Ser Ser Gly Thr Gly Gly Gly Gly Gly Gly Gly Asn Thr Gly Ser 235 240
Lys Thr Ile Val Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Asn Ile 245 250 255
Ser Leu Lys Val Asn Asn Ala Thr Ile Ala Ser Trp Thr Leu Thr Thr 260 270
Ser Met Ala Asn Tyr Thr Ala Thr Thr Ser Ala Ser Gly Gly Ser Leu 275 280 285
Val Glu Phe Thr Asn Asp Gly Gly Asn Arg Asp Val Gln Val Asp Tyr
290 295 300
Leu Ser Val Asn Gly Ala Val Arg Gln Ala Glu Asp Gln Thr Tyr 305
Thr Gly Val Tyr Gln Asn Gly Gln Cys Gly Gly Gly Asn Gly Arg Ser
Glu Trp Leu His Cys Asn Gly Ala Ile Gly Phe Gly Asn Leu
340 345 350
<210> 193
<211> 840
<212> DNA
<213> Unknown
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<223> Obtained from an environmental sample

```
<400> 193
atgacgaagt atcggttagg aataggtatt ttcattttgt tggtttgttg cttttcggcg
                                                                                                  60
gcatgtattg tgcctaaaca acaagaggaa caaaaagtgg ctcctacaga attgaccggc gcgataacat tcacagccaa cagcaacgga aacaagcccc tgaacggctc gccctacggt
                                                                                                 120
                                                                                                 180
tacgaaatat ggacacaggg cgggaccaat aacaaactga tctggttcgg gccggatcag
                                                                                                 240
ggcggcggcg cggctttcag agccgaatgg aacaacccta acgatttttt aggccgcgtg
                                                                                                 300
ggtttttact ggggtaatgg cggaaaatat accgagtaca aaaatatgta tgcggatttt
agctacacta gatctggacg caacaccgcc ggtaattatt catatatagg gatttatggc
tgggctagaa atccaaatgc cgcaaaagaa gaagacaaat tgatagagta ttatattgtg
gaagattggt ttggcaatca atggcaagag gatagctaca ccattaccac taatacaaca
                                                                                                 360
                                                                                                 420
                                                                                                 480
                                                                                                 540
agtggaaccg tattgggaag ttttactata gatggcgcgg tttataatgt cgttagaaat
                                                                                                 600
gtcagagtcc aacaaccttc gatagacgga accaaaacat tcacccaata cttcagcata cgacaaacgc cccgacagag cgggacaatt tccattaccg ggcatttcag gcaatgggag agcatgggtt tacagcttgg caatatgtac gaggcaaagt ttcttgttga agccggcggc
                                                                                                 660
                                                                                                 720
                                                                                                 780
ggcacaggat ggctggaatt ttcatacctt aaattaacga tggaagacag cttaaggtaa
                                                                                                840
<210> 194
<211> 279
 <212> PRT
<213> Unknown
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(21)
<400> 194
Met Thr Lys Tyr Arg Leu Gly Ile Gly Ile Phe Ile Leu Leu Val Cys
1 10 15
Cys Phe Ser Ala Ala Cys Ile Val Pro Lys Gln Gln Glu Glu Gln Lys
20 25 30
Val Ala Pro Thr Glu Leu Thr Gly Ala Ile Thr Phe Thr Ala Asn Ser
Asn Gly Asn Lys Pro Leu Asn Gly Ser Pro Tyr Gly Tyr Glu Ile Trp 50 55 60
Thr Gln Gly Gly Thr Asn Asn Lys Leu Ile Trp Phe Gly Pro Asp Gln 65 70 75 80
Gly Gly Ala Ala Phe Arg Ala Glu Trp Asn Asn Pro Asn Asp Phe
85 90 95
Leu Gly Arg Val Gly Phe Tyr Trp Gly Asn Gly Gly Lys Tyr Thr Glu
100 105 110
Tyr Lys Asn Met Tyr Ala Asp Phe Ser Tyr Thr Arg Ser Gly Arg Asn 115 120 125
Thr Ala Gly Asn Tyr Ser Tyr Ile Gly Ile Tyr Gly Trp Ala Arg Asn
130 135 140
Pro Asn Ala Ala Lys Glu Glu Asp Lys Leu Ile Glu Tyr Tyr Ile Val
145 150 155 160
Glu Asp Trp Phe Gly Asn Gln Trp Gln Glu Asp Ser Ser Pro Ile Thr
165 170 175
Thr Asn Thr Thr Ser Gly Thr Val Leu Gly Ser Phe Thr Ile Asp Gly
                                            185
Ala Val Tyr Asn Val Val Arg Asn Val Arg Val Gln Gln Pro Ser Ile
195 200 205
Asp Gly Thr Lys Thr Phe Thr Gln Tyr Phe Ser Ile Arg Gln Thr Pro 210 Arg Gln Ser Gly Thr Ile Ser Ile Thr Gly His Phe Arg Gln Trp Glu 225 230 235 240
Ser Met Gly Leu Gln Leu Gly Asn Met Tyr Glu Ala Lys Phe Leu Val
245 250 255
Glu Ala Gly Gly Gly Thr Gly Trp Leu Glu Phe Ser Tyr Leu Lys Leu
260 265 270
Thr Met Glu Asp Ser Leu Arg
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<210> 195 <211> 1044

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<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 195
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                                                                                              60
                                                                                            120
tccttctgga aagacagtcc gggttcagtg aatttctgca tgtactccgg cggtcgctac
                                                                                            180
acgtcgagct ggagcggcat caacaactgg gtcggcggca agggctggca aaccggatcg cgccggacca tcaactactc cggcagcttc aactcgccgg gcaatggcta cctcgcgctc tacggatgga ccaccaatcc actcgtcgag tactacatcg tcgacaactg gggcacgtat cgtccgcccg gcggccaggg ctacatgggc acggtcacga gcgacggcgc cacgtacgac
                                                                                            240
                                                                                            300
                                                                                            360
                                                                                            420
gictaicgaa cgcaacgagi cgatgcgccg tcgatcatig gigatcacca gaccitciat
                                                                                            480
                                                                                            540
caatactgga gcgtgcgtca gtcgaagagg accggcggaa ccatcaccac cggcaaccac
ttcgatggct gggcgagcta cggcatgaac ctggggaactc acaactacca gatcctggcg
accgagggtt atcaaagcag cggcagctcg gacctcaccg tgagcgaagg cagcagcagt
agcagcagcg gtggcgggag cagttcgagc agcagcggcg gcggtggcac caagagcttc
                                                                                            600
                                                                                            660
                                                                                            720
acggtccgcg cgcgcggcac ggccggtgga gagtcgatca cgttgcgcgt gaataaccag
                                                                                            780
aacgtgcaga cetggacget eggcacgage atgacgaact acaeggegte gaegtegetg
                                                                                            840
agcggcggca tcaccgtggc gttcacgaac gacggtggca accgcgatgt tcaggtggac tacatcatcg tgaacggcca gacacgccag tcggaagcgc agagctacaa caccgggctc
                                                                                            900
                                                                                            960
tacgcgaatg gacgttgcgg cggtggctcg aacagcgagt ggatgcactg caacggcgcg
                                                                                           1020
attggctacg gaaacacgcc gtaa
                                                                                           1044
<210> 196
<211> 347
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(23)
<400> 196
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Gly Val Ser Ala Ala Asn Ala Gln Thr Cys Leu Asn Ser Ser Gly Thr
20 25 30
Gly Thr Asn Asn Gly Phe Tyr Tyr Ser Phe Trp Lys Asp Ser Pro Gly 35 40 45
Ser Val Asn Phe Cys Met Tyr Ser Gly Gly Arg Tyr Thr Ser Ser Trp 50 55 60
Ser Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Gln Thr Gly Ser 65 70 75 80
Arg Arg Thr Ile Asn Tyr Ser Gly Ser Phe Asn Ser Pro Gly Asn Gly 85 90 95
Tyr Leu Ala Leu Tyr Gly Trp Thr Thr Asn Pro Leu Val Glu Tyr Tyr
100 105 110
Ile Val Asp Asn Trp Gly Thr Tyr Arg Pro Pro Gly Gly Gln Gly Tyr
115 120 125
Met Gly Thr Val Thr Ser Asp Gly Ala Thr Tyr Asp Val Tyr Arg Thr
130 140
Gln Arg Val Asp Ala Pro Ser Ile Ile Gly Asp His Gln Thr Phe Tyr
145 150 155 160
Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Thr Gly Gly Thr Ile Thr
165 170 175
Thr Gly Asn His Phe Asp Gly Trp Ala Ser Tyr Gly Met Asn Leu Gly
                                          185
Thr His Asn Tyr Gln Ile Leu Ala Thr Glu Gly Tyr Gln Ser Ser Gly
                                    200
Ser Ser Asp Leu Thr Val Ser Glu Gly Ser Ser Ser Ser Ser Gly 210 220
Gly Gly Ser Ser Ser Ser Ser Gly Gly Gly Thr Lys Ser Phe 225 230 235
Thr Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Ser Ile Thr Leu Arg
```

```
Val Asn Asn Gln Asn Val Gln Thr Trp Thr Leu Gly Thr Ser Met Thr
                                      265
              260
Asn Tyr Thr Ala Ser Thr Ser Leu Ser Gly Gly Ile Thr Val Ala Phe 275 280 285
Thr Asn Asp Gly Gly Asn Arg Asp Val Gln Val Asp Tyr Ile Ile Val 290 295 300
Asn Gly Gln Thr Arg Gln Ser Glu Ala Gln Ser Tyr Asn Thr Gly Leu
                                               315
Tyr Ala Asn Gly Arg Cys Gly Gly Gly Ser Asn Ser Glu Trp Met His 325 330 335
Cys Asn Gly Ala Ile Gly Tyr Gly Asn Thr Pro
<210> 197
<211> 636
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample
<400> 197
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                                                                                     60
ttatttgcaa caacctcaag tgcagcaacc gactattggc aaaattggac cgatggcggc
                                                                                    120
ggaacggtta atgctgtaaa cggctccggc ggtaattaca gcgtgacatg gcaaaatacc
                                                                                    180
ggaaattttg tcgtcggcaa aggctggaat accggatcgc ctaaccgaac cattaactac aatgccggcg tctgggcgcc ttccggcaat gggtatttga ctctctacgg atggacgaga aacgcactca ttgaatatta cgtcgtggat agctggggta cttatcggcc tacaggaaca
                                                                                    240
                                                                                    300
                                                                                    360
tataaaggga cggtgacaag tgatgggggc acatatgata tctatacgac catgcggcac
                                                                                    420
aacgcgcctt ccattgacgg aactcaaacg tttgcccagt actggagtgt tcgacaatcg
                                                                                    480
aaaagagcga ccggggtcaa ctcctccatt acgttcagca accacgtgaa cgcatgggct
agcaagggaa tgaatctggg aagcagctgg tcatatcagg tgttagctac agagggttat
                                                                                    540
                                                                                    600
caaagtagcg gaagctctaa cgtaacagtg tggtaa
                                                                                    636
<210> 198
<211> 211
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(28)
<400> 198
Met Phe Lys Phe Ser Lys Lys Met Met Thr Val Ile Leu Ala Ala Thr
                                          10
Met Ser Phe Gly Leu Phe Ala Thr Thr Ser Ser Ala Ala Thr Asp Tyr
20
25
30
Trp Gln Asn Trp Thr Asp Gly Gly Gly Thr Val Asn Ala Val Asn Gly
Ser Gly Gly Asn Tyr Ser Val Thr Trp Gln Asn Thr Gly Asn Phe Val
Val Gly Lys Gly Trp Asn Thr Gly Ser Pro Asn Arg Thr Ile Asn Tyr 65 70 75 80
Asn Ala Gly Val Trp Ala Pro Ser Gly Asn Gly Tyr Leu Thr Leu Tyr 85 90 95
Gly Trp Thr Arg Asn Ala Leu Ile Glu Tyr Tyr Val Val Asp Ser Trp
100 105 110
Gly Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Thr Val Thr Ser Asp
115 120 125
Gly Gly Thr Tyr Asp Ile Tyr Thr Thr Met Arg His Asn Ala Pro Ser
Ile Asp Gly Thr Gln Thr Phe Ala Gln Tyr Trp Ser Val Arg Gln Ser
150 155 160
Lys Arg Ala Thr Gly Val Asn Ser Ser Ile Thr Phe Ser Asn His Val
165 170 175
```

```
Asn Ala Trp Ala Ser Lys Gly Met Asn Leu Gly Ser Ser Trp Ser Tyr
                                             185
Gln Val Leu Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser Ser Asn Val
           195
Thr Val Trp
      210
<210> 199
<211> 1074
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 199
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                                                                                                     60
gccggcctct acatggcgcc ggcgaatgcg caaacctgca tcacgtcgag ccagacgggc accaacaacg gcaactactt ttcgttctgg aaagacagcc cgggcacggt gaacttctgc atgtactccg gcggccgcta cacgtccaac tggagcggca tcaacaactg ggtgggcggc aagggcggc agacgggctc gtcccgcacc gtctcctact ccggcagctt caattcgccg
                                                                                                   120
                                                                                                    180
                                                                                                    240
                                                                                                   300
ggtaacggct acctgacgct ctacggctgg accaccaatc cgctcatcga gtactacatc
                                                                                                   360
gtcgacaact ggggcagcta tcgtccgccg ggtggccagg gcttcatggg cacggtgaac accgacggcg gcacgtacga catctatcgc acgcaacggg tcaaccagcc gtcgatcatc ggcaccgcga cgttctacca gtactggagc gtgcggcagt cgaagcgcac cggcggcacc
                                                                                                   420
                                                                                                   480
                                                                                                   540
atcaccacgg ccaaccactt caatgcctgg gccagcctcg gcatgaacct gggacagcac
                                                                                                   600
aactaccagg tgatggccac cgagggctac cagagcagcg gcagctccga catcacggtg
                                                                                                   660
tgggaaggca cgagcagcgg cggaagcagc aatggcggca gcagcaacgg cggcagcagc
aatggtggca gcggcggcac gaagagcttc acggtgcgcg cgcgcggcac tgcgggcggc
                                                                                                   720
                                                                                                   780
gagtčcatca cgctgcgggt caacaaccag aacgtgcaga cctggacgct gggtaccagc
                                                                                                   840
atgcagaact acacggcctc gacctcgctg agcggcggca tcacggtggc gttcaccaac gacggcggca gccgcgacgt gcaggtggac tacatcatcg tgaatggcca gacccgccag tccgaacagc agagctacaa cactggcctc tacgccaatg gaagctgtgg tggcggttcg
                                                                                                   900
                                                                                                   960
                                                                                                  1020
                                                                                                  1074
aacagcgagt ggatgcattg caacggcgcc atcggctacg gcaatacgcc ctga
<210> 200
<211> 354
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(30)
<400> 200
Met Ile Phe Gly Leu Lys Ser Ile Thr Gly Arg Arg Ala Val Ala Ala
                                                  10
Leu Ala Cys Leu Ala Gly Leu Tyr Met Ala Pro Ala Asn Ala Gln Thr
20 25 30
Cys Ile Thr Ser Ser Gln Thr Gly Thr Asn Asn Gly Asn Tyr Phe Ser
Phe Trp Lys Asp Ser Pro Gly Thr Val Asn Phe Cys Met Tyr Ser Gly 50 55 60
Gly Arg Tyr Thr Ser Asn Trp Ser Gly Ile Asn Asn Trp val Gly Gly
Lys Gly Trp Gln Thr Gly Ser Ser Arg Thr Val Ser Tyr Ser Gly Ser
85 90 95
Phe Asn Ser Pro Gly Asn Gly Tyr Leu Thr Leu Tyr Gly Trp Thr Thr
100 105 110
Asn Pro Leu Ile Glu Tyr Tyr Ile Val Asp Asn Trp Gly Ser Tyr Arg
115 120 125
Pro Pro Gly Gly Gln Gly Phe Met Gly Thr Val Asn Thr Asp Gly Gly
Thr Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile Ile 145 150 155 160 Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg 165 170 175
                                                   Page 150
```

```
Thr Gly Gly Thr Ile Thr Thr Ala Asn His Phe Asn Ala Trp Ala Ser
                                                                       190
                                            185
Leu Gly Met Asn Leu Gly Gln His Asn Tyr Gln Val Met Ala Thr Glu
           195
                                      200
                                                                  205
Gly Tyr Gln Ser Ser Gly Ser Ser Asp Ile Thr Val Trp Glu Gly Thr 210 220
Ser Ser Gly Gly Ser Ser Asn Gly Gly Ser Ser Asn Gly Gly Ser Ser 225 230 235 240
Asn Gly Gly Ser Gly Gly Thr Lys Ser Phe Thr Val Arg Ala Arg Gly
245 250 255
Thr Ala Gly Gly Glu Ser Ile Thr Leu Arg Val Asn Asn Gln Asn Val
Gln Thr Trp Thr Leu Gly Thr Ser Met Gln Asn Tyr Thr Ala Ser Thr
275 280 285
Ser Leu Ser Gly Gly Ile Thr Val Ala Phe Thr Asn Asp Gly Gly Ser 290 295 300
Arg Asp Val Gln Val Asp Tyr Ile Ile Val Asn Gly Gln Thr Arg Gln 315 320
Ser Glu Gln Gln Ser Tyr Asn Thr Gly Leu Tyr Ala Asn Gly Ser Cys
325 330 335
Gly Gly Gly Ser Asn Ser Glu Trp Met His Cys Asn Gly Ala Ile Gly
Tyr Gly
<210> 201
<211> 1002
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 201
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                                                                                                  60
                                                                                                120
ggggcggcgg tcaatcctgt gacgatcgag atgcaaaaac agttgttgat cgatcatgtc
aacagtatta cggcagagaa ccatatgaag tttgagcatc ttcagccgga agaagggaaa
                                                                                                180
tttacctttc aggaagcgga tcggattgtg gattttgctt gttcgcaccg aatggcggttcgagggcaca cacttgtatg gcacaaccag actccggatt gggtgttca agatggtcaa
                                                                                                240
                                                                                                300
ggccatttcg tcagtcggga tgtgttgctt gagcggatga aatgtcacat ttcaactgtt
gtacggcgat acaagggaaa aatatattgt tgggatgtca tcaacgaagc ggtagccgac
                                                                                                360
                                                                                                420
gaaggagacg aattgttgag gccgtcgaag tggcgacaaa tcatcgggga cgatttatg
gaacaagcat ttctctacgc ttatgaagct gacccagatg cactgctttt ttacaatgac
tataatgaat gttttccgga aaagagagaa aaaatttttg cacttgtcaa atcgctgcgt
                                                                                                480
                                                                                                540
                                                                                                600
gataaaggca ttccgattca tggcatcggc atgcaggcgc actggagcct gacccgcccg
                                                                                                660
tcgcttgatg aaattcgtgc ggcgattgaa cggtatgcgt cccttggtgt tgttcttcat attacggaac tcgatgtatc catgtttgaa tttcacgatc gtcgaaccga tttggctgtc ccgacgaacg aaatgatcga acagcaagca gaacggtatg ggcaaatttt tgctttgttt
                                                                                                720
                                                                                                780
                                                                                                840
aaggagtatc gcgatgttat tcaaagtgtc acattttggg gaattgctga tgaccataca tggctcgata actttccagt gcacgggaga aaaaactggc cgcttttgtt cgatgaacag
                                                                                                900
                                                                                                960
cataaaccga aaccagcttt ttggcgggca gtgagtgtct ga
                                                                                               1002
<210> 202
<211> 333
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 202
Met Lys Met Asn Ser Ser Leu Pro Ser Leu Arg Asp Val Phe Ala Asn
1 10 15
Asp Phe Arg Ile Gly Ala Ala Val Asn Pro Val Thr Ile Glu Met Gln 20 25 30
Lys Gln Leu Leu Ile Asp His Val Asn Ser Ile Thr Ala Glu Asn His 35

Met Lys Phe Glu His Leu Gln Pro Glu Glu Gly Lys Phe Thr Phe Gln 50
```

```
Glu Ala Asp Arg Ile Val Asp Phe Ala Cys Ser His Arg Met Ala Val
                                                                                                     75
Arg Gly His Thr Leu Val Trp His Asn Gln Thr Pro Asp Trp Val Phe
 Gln Asp Gly Gln Gly His Phe Val Ser Arg Asp Val Leu Leu Glu Arg
Met Lys Cys His Ile Ser Thr Val Val Arg Arg Tyr Lys Gly Lys Ile
115 120 125
 Tyr Cys Trp Asp Val Ile Asn Glu Ala Val Ala Asp Glu Gly Asp Glu
130 135 140
 Leu Leu Arg Pro Ser Lys Trp Arg Gln Ile Ile Gly Asp Asp Phe Met
145 150 155 160
Glu Gln Ala Phe Leu Tyr Ala Tyr Glu Ala Asp Pro Asp Ala Leu Leu
165 170 175
Phe Tyr Asn Asp Tyr Asn Glu Cys Phe Pro Glu Lys Arg Glu Lys Ile
180 185 190
Phe Ala Leu Val Lys Ser Leu Arg Asp Lys Gly Ile Pro Ile His Gly 200 205
Ile Gly Met Gln Ala His Trp Ser Leu Thr Arg Pro Ser Leu Asp Glu
210 220 220
Ile Arg Ala Ala Ile Glu Arg Tyr Ala Ser Leu Gly Val Val Leu His 225 230 235 240
Ile Thr Glu Leu Asp Val Ser Met Phe Glu Phe His Asp Arg Arg Thr
Asp Leu Ala Val Pro Thr Asn Glu Met Ile Glu Gln Gln Ala Glu Arg
Tyr Gly Gln Ile Phe Ala Leu Phe Lys Glu Tyr Arg Asp Val Ile Gln 275 280 285
Ser Val Thr Phe Trp Gly Ile Ala Asp Asp His Thr Trp Leu Asp Asn 290 295 300
Phe Pro Val His Gly Arg Lys Asn Trp Pro Leu Leu Phe Asp Glu Gln 305 310 315
His Lys Pro Lys Pro Ala Phe Trp Arg Ala Val Ser Val
<210> 203
<211> 687
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 203
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                                                                                                                                                                                 60
ctycacting categorial titiggingly claicacyca tacticing cycactingly ctycactingly ctycactingly ctycactingly category cocceptor cycactingly catalogs cocceptor cycactingly gazgycogca cycoctycat gazgycogca cycoctycat gazycogcy catalogy gazycogca attatagorial caccalogy gazycogca cycoctycat cycactycy catalogy cycactycy cy
                                                                                                                                                                               120
                                                                                                                                                                               180
                                                                                                                                                                               240
                                                                                                                                                                               300
                                                                                                                                                                               360
                                                                                                                                                                               420
                                                                                                                                                                               480
ccttcgatca tcggcaacgc cacgttctac caatattgga gcgtgcgcac ttcgcgccgc gggcaaggca cgaacaacac gatcaccttc gccaatcacg tcaacgcttg gcgcagccgc ggcatgaacc ttgggaccat gaattatcaa gtcatggcca cggaaggttt cggctcgaac
                                                                                                                                                                               540
                                                                                                                                                                               600
                                                                                                                                                                               660
ggaagctcca acctcacagt atggtag
                                                                                                                                                                               687
<210> 204
<211> 228
<212> PRT
<213> Unknown
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(30)
<400> 204
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Met Lys Ser Ala Arg Ala Leu Leu Val Ala Leu Ser Arg Ile Leu Pro
                                                  10
Ile Ala Leu Val Leu Leu Leu Ala Pro Val Pro Ala Gln Ala Gln Gln 20 25 30
Val Cys Asn Asn Gly Thr Gly Thr His Asn Gly Phe Phe Trp Thr Phe 35 40 45
Trp Lys Asp Gly Gly Thr Ala Cys Met Thr Leu Gly Ser Gly Gly Asn 50 60
Tyr Ser Thr Thr Phe Asn Leu Ser Gly Gly Arg Asn Leu Val Ala Gly 65 70 75 80
Lys Gly Trp Gln Thr Gly Ser Thr Asn Arg Val Val Gly Tyr Asn Ala
                      85
                                                  90
Gly Val Trp Asn Pro Gly Thr Asn Ser Tyr Leu Thr Leu Tyr Gly Trp
100 105 110
Ser Thr Asn Pro Leu Val Glu Tyr Tyr Val Val Asp His Trp Gly Ser
115 120 125
Gln Phe Thr Pro Pro Gly Asn Gly Ala Gln Ser Met Gly Thr Val Thr
                                 135
Thr Asp Gly Gly Thr Tyr Asn Ile Tyr Arg Thr Gln Arg Val Asn Ala
145 150 160
Pro Ser Ile Ile Gly Asn Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg
165 170 175
Thr Ser Arg Arg Gly Gln Gly Thr Asn Asn Thr Ile Thr Phe Ala Asn
180 185 190
His Val Asn Ala Trp Arg Ser Arg Gly Met Asn Leu Gly Thr Met Asn
195 200 205
Tyr Gln Val Met Ala Thr Glu Gly Phe Gly Ser Asn Gly Ser Ser Asn
210 215 220
Leu Thr Val Trp
225
<210> 205
<211> 1068
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample
<400> 205
atgcaaattt tcaaatcacc actgtcatgg gccggatcac tattactgat cctgtccacc
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gccctgtttt caacagcggc cactgcccag gaatactgct ccaaccagac cggtacacac agcggttttt actttaccca ttggtctgac ggcggcggta ctgcctgcat tactctggga gacgacggaa attacagtta cacctggtcc aacacaggca attttgtcgg tggcaagggc
                                                                                                 120
                                                                                                 180
                                                                                                 240
tggagtaccg gcacctccaa tcgggtgatc ggttacaacg ccggagacta ctcgcctcc ggcaactcct acctggcgct gtatggctgg agcaccaatc cactgattga gtactacgtg gtggatagct ggggtagctg gcgtccgccg ggtggcacct cggtaggtac agtcaccagc gatggcggga cttacgacct gtaccgcacc gagcgcgtgc agcagccct catcgaaggc
                                                                                                 300
                                                                                                 360
                                                                                                 420
                                                                                                 480
acggccacct tctatcaata ttggagcgtg cgcacctcac agcgtcccca ggggcagaac
                                                                                                 540
aacaccatca cettteagaa ceaegtggat geetgggeea ateagggetg gaacetegge
                                                                                                 600
acccacaact atcaggtaat ggcgaccgaa ggctacgaaa gcagcggcag ctccaacgtcacggtttggg attccggcac cagtagcggt aacggtggca acgctggcgg cggtggtggc
                                                                                                 660
                                                                                                 720
gaggcaggta acggctccaa ctcactggtc gtgcgtgcgg tgggcacttc gggcaacgaa
                                                                                                 780
cagttgcgcg tcaacgtcag cggcaacacg gttgaaaccc tgaacctgtc taccaactgg
                                                                                                 840
caggactaca ccatcaacac caacgcttcc ggcgatgtga atgtggagtt gatcaacgat cagggcgagg gctacgaagc ccgggtggaa tacgtcatcg tcaacggcga tacccgctac ggcgctgatc agagctacaa caccagcgcc tgggacggcg agtgcggcgg cggttccttt
                                                                                                 900
                                                                                                 960
                                                                                                1020
accatgtgga tgcactgcga aggcatcctc ggttttggcg atatgtaa
                                                                                                1068
<210> 206
<211> 355
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL <222> (1)...(29)
```

<400> 206

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Met Gln Ile Phe Lys Ser Pro Leu Ser Trp Ala Gly Ser Leu Leu
1 10 15
                                              10
Ile Leu Ser Thr Ala Leu Phe Ser Thr Ala Ala Thr Ala Gln Glu Tyr 20 25 30
Cys Ser Asn Gln Thr Gly Thr His Ser Gly Phe Tyr Phe Thr His Trp
Ser Asp Gly Gly Gly Thr Ala Cys Ile Thr Leu Gly Asp Asp Gly Asn
50
60
Tyr Ser Tyr Thr Trp Ser Asn Thr Gly Asn Phe Val Gly Gly Lys Gly 65 75 80
Trp Ser Thr Gly Thr Ser Asn Arg Val Ile Gly Tyr Asn Ala Gly Asp
85
90
95
Tyr Ser Pro Ser Gly Asn Ser Tyr Leu Ala Leu Tyr Gly Trp Ser Thr 100 - 105 - 110
Asn Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly Ser Trp Arg
Pro Pro Gly Gly Thr Ser Val Gly Thr Val Thr Ser Asp Gly Gly Thr
130 140
Tyr Asp Leu Tyr Arg Thr Glu Arg Val Gln Gln Pro Ser Ile Glu Gly
145 150 155
Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Thr Ser Gln Arg Pro
165 170 175
Gln Gly Gln Asn Asn Thr Ile Thr Phe Gln Asn His Val Asp Ala Trp
Ala Asn Gln Gly Trp Asn Leu Gly Thr His Asn Tyr Gln Val Met Ala
Thr Glu Gly Tyr Glu Ser Ser Gly Ser Ser Asn Val Thr Val Trp Asp
Ser Gly Thr Ser Ser Gly Asn Gly Gly Asn Ala Gly Gly Gly Gly 225 235 240
Glu Ala Gly Asn Gly Ser Asn Ser Leu Val Val Arg Ala Val Gly Thr
245 250 255
Ser Gly Asn Glu Gln Leu Arg Val Asn Val Ser Gly Asn Thr Val Glu 260 270
Thr Leu Asn Leu Ser Thr Asn Trp Gln Asp Tyr Thr Ile Asn Thr Asn 275 280 285
Ala Ser Gly Asp Val Asn Val Glu Leu Ile Asn Asp Gln Gly Glu Gly 290 _ _ _ 295 _ _ 300
Tyr Glu Ala Arg Val Glu Tyr Val Ile Val Asn Gly Asp Thr Arg Tyr 305 310 315 320
Gly Ala Asp Gln Ser Tyr Asn Thr Ser Ala Trp Asp Gly Glu Cys Gly 325 330 335
Gly Gly Ser Phe Thr Met Trp Met His Cys Glu Gly Ile Leu Gly Phe 340
Gly Asp Met
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<211> 633
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
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                                                                                          120
acggtgaatg cggttaacgg gtccgggggc aattacagcg taacttggca aaatagcggg
aacttcgtgg tcggcaaagg ctggagcgta gggtcgccaa atcggacgat caattacaat
                                                                                         180
240
300
gccggcatct gggaaccttc ggggaacggg tacttgaccc tttacggatg gactagaaac tcgctgatcg agtattacgt tgtcgacagt tgggggacgt accggccaac aggtactcac aaaggaacgg tgaacagcga cggaggcacc tacgatattt atacgaccat gcgctataat gcgccttcca ttgatggcac gcagacgttc caacagttct ggagcgtgcg gcaatcgaaa cgaccaaccg gcagcaacgt ctccatcaacca accggaatgc ctggagaag
                                                                                          360
                                                                                          420
                                                                                          480
                                                                                         540
aagggcatga acctgggcag cagctggtcg taccaggtct tggcgacgga aggctatcag
                                                                                          600
agcagcggaa gatccaacgt cacggtgtgg taa
                                                                                         633
                                               Page 154
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<210> 208
<211> 210
<212> PRT
<213> Unknown
<220>
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<221> SIGNAL
<222> (1)...(27)
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Ser Phe Gly Leu Phe Gly Ala Thr Ser Ser Ala Ala Thr Asp Tyr Trp
Gln Tyr Trp Thr Asp Gly Gly Gly Thr Val Asn Ala Val Asn Gly Ser
45
Gly Gly Asn Tyr Ser Val Thr Trp Gln Asn Ser Gly Asn Phe Val Val
Gly Lys Gly Trp Ser Val Gly Ser Pro Asn Arg Thr Ile Asn Tyr Asn 65 75 80
Ala Gly Ile Trp Glu Pro Ser Gly Asn Gly Tyr Leu Thr Leu Tyr Gly
85 90 _____ 95
Trp Thr Arg Asn Ser Leu Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly
100 105 110
Thr Tyr Arg Pro Thr Gly Thr His Lys Gly Thr Val Asn Ser Asp Gly
115 120 125
Gly Thr Tyr Asp Ile Tyr Thr Thr Met Arg Tyr Asn Ala Pro Ser Ile
130 140
Asp Gly Thr Gln Thr Phe Gln Gln Phe Trp Ser Val Arg Gln Ser Lys
145 150 155 160
Arg Pro Thr Gly Ser Asn Val Ser Ile Thr Phe Ser Asn His Val Asn 165 170 175
Ala Trp Arg Ser Lys Gly Met Asn Leu Gly Ser Ser Trp Ser Tyr Gln
180 185 190
Val Leu Ala Thr Glu Gly Tyr Gln Ser Ser Gly Arg Ser Asn Val Thr
195 200 205
Val Trp
      210
<210> 209
<211> 1194
<212> DNA
<213> Unknown
<220>
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                                                                                                             120
attacttcga accaaactgg taaaatcggc gatatcggtt acgaactctg ggacgaaaac ggtcatggtg gtagtgctac cttctatagc gatggttcca tggactgcaa tatcactggt gctaaggact atctctgccg tgcgggcctt tccctcggca gtaacaagac ctacaaggaa
                                                                                                             180
                                                                                                             240
                                                                                                             300
cttggtggtg atatgattgc cgagttcaag cttgtgaaga gcggtgccca gaatgtgggt
                                                                                                             360
tactcttata tcggtatcta tggctggatg gaaggtgttt ctggaacgcc tagccagttg
gtcgaatact acgtgattga taacaccctc gccaatgaca tgccgggtag ctggattggt
aacgaaagaa agggtaccat tacggttgac ggcggtacct atactgttta tcgcaatacc
Cgtacaggtc cggctattag gaacagcggt aacgtattag cacacagta ttcagggt
                                                                                                             420
                                                                                                             480
                                                                                                             540
                                                                                                             600
Cgtacctctc cgcgcgattg Cggtaccatc aatatttccg aacacatgag acagtgggaa aagatgggca tgaccatggg taagctctac gaagccaagg tgcttggcga agcgggtaac gtgaatggcg aagtccgcgg tggtcacatg gacttcccgc atgctaaggt ttatgtgaaa aacggccttg accggcgt tcctcttct gtgaagtcca gctctctac agtaacgcca
                                                                                                             660
                                                                                                            720
                                                                                                             780
                                                                                                             840
aaatccagct cctcgaaggg taacggcaac gtttctggta aaattgacgc ctgcaaggac gctatgggcc atgaaggcaa agaaacgaga actcagggtc agaacaactc tagcgtgacg ggtaacgtcg gcagctctcc gtaccactat gaaatttggt atcagggtgg taacaactcc atgacgttct acgacaacgg tacttataag gcaagctggt atggtaccaa cgacttcctt
                                                                                                             900
                                                                                                            960
                                                                                                           1020
                                                                                                           1080
                                                         Page 155
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1140

1194

gctcgtgtcg gtttcaagta tgatgaaaag cacacttacg aagaacttgg ccctatcgat gcctactaca agtggagcaa gcagggtagt gctggtggct acaactacat cggt <210> 210 <211> 398 <212> PRT <213> Unknown <223> Obtained from an environmental sample <221> SIGNAL <222> (1)...(25) <400> 210 Met Lys Thr Phe Ser Val Thr Lys Ser Ser Val Val Phe Ala Met Ala 1 5 10 15 Leu Gly Met Ala Ser Thr Ala Phe Ala Gln Asp Phe Cys Ser Asn Ala 20 25 30 Gln His Ser Gly Gln Lys Val Thr Ile Thr Ser Asn Gln Thr Gly Lys
45
40
45 Ile Gly Asp Ile Gly Tyr Glu Leu Trp Asp Glu Asn Gly His Gly Gly 50 55 _ 60 Ser Ala Thr Phe Tyr Ser Asp Gly Ser Met Asp Cys Asn Ile Thr Gly 70 75 80 Ala Lys Asp Tyr Leu Cys Arg Ala Gly Leu Ser Leu Gly Ser Asn Lys 85 90 95 Thr Tyr Lys Glu Leu Gly Gly Asp Met Ile Ala Glu Phe Lys Leu Val Lys Ser Gly Ala Gln Asn Val Gly Tyr Ser Tyr Ile Gly Ile Tyr Gly
115 120 125 Trp Met Glu Gly Val Ser Gly Thr Pro Ser Gln Leu Val Glu Tyr Tyr 130 140 Val Ile Asp Asn Thr Leu Ala Asn Asp Met Pro Gly Ser Trp Ile Gly
145 150 155 160 Asn Glu Arg Lys Gly Thr Ile Thr Val Asp Gly Gly Thr Tyr Thr Val Tyr Arg Asn Thr Arg Thr Gly Pro Ala Ile Lys Asn Ser Gly Asn Val Thr Phe Tyr Gln Tyr Phe Ser Val Arg Thr Ser Pro Arg Asp Cys Gly
195
200
205 Thr Ile Asn Ile Ser Glu His Met Arg Gln Trp Glu Lys Met Gly Met 210 220 Thr Met Gly Lys Leu Tyr Glu Ala Lys Val Leu Gly Glu Ala Gly Asn 235 240 Val Asn Gly Glu Val Arg Gly Gly His Met Asp Phe Pro His Ala Lys
245 _ 250 255 Val Tyr Val Lys Asn Gly Ser Asp Pro Ala Ser Ser Ser Ser Val Lys
260 265 270 Ser Ser Ser Ser Thr Val Thr Pro Lys Ser Ser Ser Ser Lys Gly Asn 275 280 285 Gly Asn Val Ser Gly Lys Ile Asp Ala Cys Lys Asp Ala Met Gly His 290 295 300 Glu Gly Lys Glu Thr Arg Thr Gln Gly Gln Asn Asn Ser Ser Val Thr 305 310 315 320 Gly Asn Val Gly Ser Ser Pro Tyr His Tyr Glu Ile Trp Tyr Gln Gly 325 330 335 Gly Asn Asn Ser Met Thr Phe Tyr Asp Asn Gly Thr Tyr Lys Ala Ser 340 345 350 Trp Asn Gly Thr Asn Asp Phe Leu Ala Arg Val Gly Phe Lys Tyr Asp 355 360 365 Glu Lys His Thr Tyr Glu Glu Leu Gly Pro Ile Asp Ala Tyr Tyr Lys
370
375
380 Trp Ser Lys Gln Gly Ser Ala Gly Gly Tyr Asn Tyr Ile Gly 385 395 <210> 211 <211> 1086 <212> DNA

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                                                                                       120
aacggttttt actttaccca ttggtcagac ggtggcggta ccgcctgcat gactctgggg
                                                                                       180
gacgacggca actacagcta tacctggtcc aacactggca attttgtcgg tggtaagggc
                                                                                       240
tggagcacag gtacatccaa ccgggtgatt ggttacaacg ccggagacta ctcgcctcc ggcaactcct acctggcact gtatggctgg agcaccaatc cgctgattga atattacgtg
                                                                                       300
                                                                                      360
gtcgacagtt ggggcagctg gcgtccgccg ggtggcacct ctgtgggcac ggtaaccagc
                                                                                       420
gacggtggca cttacgacct gtaccgaacc cagcgtgtgc agcagccctc cattgagggt
                                                                                      480
acggccacct tctatcaata ctggagcgtg cgcacctcac agcggcctca ggggcaaaac aacaccatca cctttcagaa ccacgtgaat gcctgggcca atcagggctg gaatctgggc
                                                                                      540
                                                                                      600
acccacaact atcaggtgat ggcgaccgaa ggctacgaaa gcagcggcag ctccaacgtc
                                                                                      660
acceptitigg attecggeac cagtageggt ggeggtggeg gtggeacge ggggggggggggggggggggggeggeeccccg gtggtggtga ggetggagge ggetecaact cactggttgt gegtgeggtg ggeacttegg geaatgaaca gttgeggte aacgteagtg geaacacggt ggaaaccetg aacctgteta ceaactggea ggactacace ateaacacea aegeeteegg egatgteaat
                                                                                      720
                                                                                      780
                                                                                      840
                                                                                      900
gtggaattga tcaacgacca gggcgaaggc tacgaggccc gcgtcgagta cgtcatcatc
                                                                                      960
aacggcgata cccgctacgg cgccgaccag agctacaaca ccagcgcctg ggacggcgag
tgcggtagcg gttcctttac catgtggatg cactgcgaag gcatcctcgg ttttggcgat
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                                                                                     1080
atgtaa
                                                                                     1086
<210> 212
<211> 361
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(29)
<400> 212
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1 5 10
Ala Leu Ser Ala Val Leu Leu Ser Ala Thr Ala Ser Ala Gln Gln His
20 25 30
Cys Ser Asn Gln Thr Gly Thr His Asn Gly Phe Tyr Phe Thr His Trp
Ser Asp Gly Gly Gly Thr Ala Cys Met Thr Leu Gly Asp Asp Gly Asn 50 60
Tyr Ser Tyr Thr Trp Ser Asn Thr Gly Asn Phe Val Gly Gly Lys Gly 65 75 80
Trp Ser Thr Gly Thr Ser Asn Arg Val Ile Gly Tyr Asn Ala Gly Asp 85 90 95
Tyr Ser Pro Ser Gly Asn Ser Tyr Leu Ala Leu Tyr Gly Trp Ser Thr
Asn Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly Ser Trp Arg
115 120 125
Pro Pro Gly Gly Thr Ser Val Gly Thr Val Thr Ser Asp Gly Gly Thr
                             135
Tyr Asp Leu Tyr Arg Thr Gln Arg Val Gln Gln Pro Ser Ile Glu Gly
                        150
                                                 155
Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Thr Ser Gln Arg Pro
165 170 175
Gln Gly Gln Asn Asn Thr Ile Thr Phe Gln Asn His Val Asn Ala Trp
180
185
190
Ala Asn Gln Gly Trp Asn Leu Gly Thr His Asn Tyr Gln Val Met Ala
195 200 205
Thr Glu Gly Tyr Glu Ser Ser Gly Ser Ser Asn Val Thr Val Trp Asp 210 220
Gly Ala Pro Gly Gly Glu Ala Gly Gly Gly Ser Asn Ser Leu Val
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Val Arg Ala Val Gly Thr Ser Gly Asn Glu Gln Leu Arg Val Asn Val 260 270
 Ser Gly Asn Thr Val Glu Thr Leu Asn Leu Ser Thr Asn Trp Gln Asp 285
 Tyr Thr Ile Asn Thr Asn Ala Ser Gly Asp Val Asn Val Glu Leu Ile
290 295 300
 Asn Asp Gln Gly Glu Gly Tyr Glu Ala Arg Val Glu Tyr Val Ile Ile
305 310 315 320
Asn Gly Asp Thr Arg Tyr Gly Ala Asp Gln Ser Tyr Asn Thr Ser Ala
325 330 335
Trp Asp Gly Glu Cys Gly Ser Gly Ser Phe Thr Met Trp Met His Cys 340 345 350
Glu Gly Ile Leu Gly Phe Gly Asp Met 355
 <210> 213
 <211> 912
 <212> DNA
 <213> Unknown
 <223> Obtained from an environmental sample
 <400> 213
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tctttctgga aggactccgg caatgcgtcc ttcactctct acgatggcgg acgttacggc tcgcaatgga atagcggcac caacaattgg gtgggcggta aaggctggaa cccgggcggc gcaaaagtcg ttaactacga aggttattac ggcgttaaca attcccagaa ttcttacctg
                                                                                                       120
                                                                                                       180
                                                                                                       240
ğcactctacg ggtggacccg caatccgctg atcgagtact acataatcga aagttacggt
                                                                                                       300
tcgtacaacc catcgagctg tagtggcggt actaactacg gtagcttcca aagcgatggt gcgacctata acgtccgccg ttgccagcgc gtacagcagc catcgattga tggaacgcaa acgttctatc agtatttcag cgttcgctca cccaaaaagg gcttcggcca aatcagcggc actatcaatg taggcaacca ctttaattat tgggccagca aagggctgaa tttgggtagc
                                                                                                       360
                                                                                                       420
                                                                                                       480
                                                                                                       540
cacgattacă tggttctggc gactgaaggc tatcagagca gcggcaattc agatatttcc
                                                                                                       600
gtgtccgaag gcagcagcgg cggctcttcc tcaggcggtt cgacctccag cggaagctcc tccggtagta cgaccagttc ttcaggaggc ggtggcggcg gcatcacagt acgtgctcgc ggcactaatg gtgatgagcg tatcagcctg cgtgtcggcg gttctgcggt agccagttgg acactcagta ccagcgcaca aagctatagc tacacaggcg gcgctctgcg cgataccag
                                                                                                       660
                                                                                                       720
                                                                                                       780
                                                                                                       840
gtggaattcg atatcaagct tatcgatacc gtcgacctcg agggggggcc cggtacccaa
                                                                                                       900
ttcgccctat ag
                                                                                                       912
<210> 214
<211> 303
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
Val Asn Ala Gln Gln Thr Leu Thr Ser Asn Ser Thr Gly Thr His Gly
1 10 15
Gly His Tyr Tyr Ser Phe Trp Lys Asp Ser Gly Asn Ala Ser Phe Thr
Leu Tyr Asp Gly Gly Arg Tyr Gly Ser Gln Trp Asn Ser Gly Thr Asn 35 40 45
Asn Trp Val Gly Gly Lys Gly Trp Asn Pro Gly Gly Ala Lys Val Val
Asn Tyr Glu Gly Tyr Tyr Gly Val Asn Asn Ser Gln Asn Ser Tyr Leu 70 75 80
Ala Leu Tyr Gly Trp Thr Arg Asn Pro Leu Ile Glu Tyr Tyr Ile Ile
85 90 95
Glu Ser Tyr Gly Ser Tyr Asn Pro Ser Ser Cys Ser Gly Gly Thr Asn 100 110
Tyr Gly Ser Phe Gln Ser Asp Gly Ala Thr Tyr Asn Val Arg Arg Cys
115
120
125
Gln Arg Val Gln Gln Pro Ser Ile Asp Gly Thr Gln Thr Phe Tyr Gln
130
140
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```
Tyr Phe Ser Val Arg Ser Pro Lys Lys Gly Phe Gly Gln Ile Ser Gly
                             150
                                                          155
Thr Ile Asn Val Gly Asn His Phe Asn Tyr Trp Ala Ser Lys Gly Leu
165 170 175
Asn Leu Gly Ser His Asp Tyr Met Val Leu Ala Thr Glu Gly Tyr Gln
180 185 190
Ser Ser Gly Asn Ser Asp Ile Ser Val Ser Glu Gly Ser Ser Gly Gly
195 200 205
Ser Ser Ser Gly Gly Ser Thr Ser Ser Gly Ser Ser Gly Ser Thr
210 215 220
Thr Ser Ser Ser Gly Gly Gly Gly Gly Gly Ile Thr Val Arg Ala Arg 225 230 240
Gly Thr Asn Gly Asp Glu Arg Ile Ser Leu Arg Val Gly Gly Ser Ala 245 250 255
Val Ala Ser Trp Thr Leu Ser Thr Ser Ala Gln Ser Tyr Ser Tyr Thr
Gly Gly Ala Ser Gly Asp Ile Gln Val Glu Phe Asp Ile Lys Leu Ile
275 280 285
Asp Thr Val Asp Leu Glu Gly Gly Pro Gly Thr Gln Phe Ala Leu 290 295 300
<210> 215
<211> 1065
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 215
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                                                                                                     60
                                                                                                    120
                                                                                                    180
                                                                                                    240
aagggctgga atccgggcgg tcgtcggacc gtcacctatt cggggacgtt caacccgaac
                                                                                                    300
ggcaatteet ateteaeget gtaeggetgg accaecaate caetggtega gtaetaeate
                                                                                                    360
gtcgacagct ggggcagctg gcgtccgccg ggttccggct acatgggttc cgtcacgagc gacggcggca cctacgacat ctatcgcacg cagcgcgtca accagccctc gatcatcggc accgcgacgt tctaccagta ctggagcgtg cggcagcaga agcgcgtggg tggcaccatc accaccggca accacttcga tgcctgggct tcgctgggca tgaacctcgg ccagcacaac
                                                                                                    420
                                                                                                    480
                                                                                                    540
                                                                                                    600
tacatggtca tggccaccga gggctaccag agcagcggca gctccgacat cacggtgggc
ggcaccagca gctcctcgtc gtcgagcggg ggcagcagca gcagtagcag cagcagcggg
ggtggcggct cgaagagctt caccgtgcgc gcgcggggtt cgacgggcgg tgagcagatc
                                                                                                    660
                                                                                                    720
                                                                                                    780
agtttgcgcg tgaacaacca gaccgtgcag aactggacgc tgggcaccag catgcagaac
                                                                                                    840
tacaccgcgt ccaccaacct gagcggcggc atcaccgtgc acticaccaa tgacagcggc
                                                                                                    900
aaccgcgacg tgcaggtgga ctacatccag gtgaacggcc agacgcgtca atccgagcag cagagctaca acaccgggct gtatgccaac ggcagctgtg gcggcggcgg ctacagcgag
                                                                                                    960
                                                                                                  1020
tggatgcatt gcaatggcgc gatcggttac ggcaacacgc cgtag
                                                                                                  1065
<210> 216
<211> 354
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(31)
<400> 216
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Ala Leu Ala Gly Leu Ala Leu Leu Gly Thr Ala Pro Ala Asn Ala Gln
20 25 30
Thr Cys Leu Thr Asn Asn Ser Thr Gly Thr Asn Asn Gly Tyr Tyr Tyr 35 40 45
Ser Phe Trp Lys Asp Ser Gly Asn Val Thr Phe Cys Met Tyr Gly Gly 50 60
```

```
Gly Arg Tyr Thr Ser Gln Trp Ser Asn Ile Asn Asn Trp Val Gly Gly
65 70 75 80
  Lys Gly Trp Asn Pro Gly Gly Arg Arg Thr Val Thr Tyr Ser Gly Thr
85 90 95
  Phe Asn Pro Asn Gly Asn Ser Tyr Leu Thr Leu Tyr Gly Trp Thr Thr
 Asn Pro Leu Val Glu Tyr Tyr Ile Val Asp Ser Trp Gly Ser Trp Arg
  Pro Pro Gly Ser Gly Tyr Met Gly Ser Val Thr Ser Asp Gly Gly Thr
130 140
 Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn Gln Pro Ser Ile Ile Gly
                                                150
 Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val Arg Gln Gln Lys Arg Val
165 170 175
 Gly Gly Thr Ile Thr Thr Gly Asn His Phe Asp Ala Trp Ala Ser Leu
180 185 190
 Gly Met Asn Leu Gly Gln His Asn Tyr Met Val Met Ala Thr Glu Gly
195 200 205
 Tyr Gln Ser Ser Gly Ser Ser Asp Ile Thr Val Gly Gly Thr Ser Ser 210 220
 Ser Ser Ser Ser Ser Gly Gly Ser Ser Ser Ser Ser Ser Ser Ser Gly 235 240
 Gly Gly Gly Ser Lys Ser Phe Thr Val Arg Ala Arg Gly Ser Thr Gly
245 250 255
 Gly Glu Gln Ile Ser Leu Arg Val Asn Asn Gln Thr Val Gln Asn Trp
 Thr Leu Gly Thr Ser Met Gln Asn Tyr Thr Ala Ser Thr Asn Leu Ser 275 280 285
 Gly Gly Ile Thr Val His Phe Thr Asn Asp Ser Gly Asn Arg Asp Val
 Gln Val Asp Tyr Ile Gln Val Asn Gly Gln Thr Arg Gln Ser Glu Gln 305 310 315 320
 Gln Ser Tyr Asn Thr Gly Leu Tyr Ala Asn Gly Ser Cys Gly Gly Gly 325
 Gly Tyr Ser Glu Trp Met His Cys Asn Gly Ala Ile Gly Tyr Gly Asn
 Thr Pro
 <210> 217
 <211> 1083
 <212> DNA
 <213> Unknown
 <220>
 <223> Obtained from an environmental sample
 <400> 217
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                                                                                                                                                                       60
 ggcctctaca tggcgccggc aaacgcgcaa acctgcatca cgtccagcca gaccggcacc
                                                                                                                                                                     120
aacaacggga actacttttc gttctggaaa gacagcccgg gcacggtgaa cttctgcatg tacccgaatg gccgctacac ctcgaactgg agcggcatca acaactgggt cggcggcaag ggctggtcga ccggctccag ccgcaccgtc agctattcgg gcagcttcaa ttcgcccggc aacggctacc tgactctcta cgggtggacc accaacccgc tcatcgagta ctacatcgtc gagaactggg gtaactaccg cccgccggc ggccaggggt acatggggac cgtcaattcc gacggggcga cctatgacat ctaccggacc ttccggggaca accagcctg catcacggg aactcctgga acttctacca gtactggacc atgcgcagt ccaagcgca accagcgcag acttctacca gtactggacc atgcgccagt ccaagcgcag acctctaca gtactggacc atgcgccagt ccaagcgcag acctcctaca gtactggacc accaacccag accagcacag accag
                                                                                                                                                                     180
                                                                                                                                                                     240
                                                                                                                                                                     300
                                                                                                                                                                     360
                                                                                                                                                                     420
                                                                                                                                                                     480
 aactcctgcg acttctacca gtactggagc gtgcgccagt ccaagcgcag cagcggcacc
                                                                                                                                                                     540
atcaccacgg ccaatcactt cgcggcgtgg aacagcctcg gcatgaacct gggccagcac aactaccagg tcatggccac cgagggttac cagagcagcg gcagctccga catcacggtc acggaaggcg gcggcggcag cagcaatggt ggcagcagca acggcggcag cagcaatggc
                                                                                                                                                                     600
                                                                                                                                                                    660
                                                                                                                                                                    720
ggcagcagca atggcggcgg cggcggcacc aagagcttca cggtccgcgc ccgtggcacc
                                                                                                                                                                    780
gcgggtggcg agtccatcac gctgcgtgtc aacaaccaga acgtgcagac ctggacgctg
ggcaccggca tgcagaacta cacggcctcg acctcgctga gcggtggcat cacggtgcac
ttcaccaacg acggcggaag ccgcgacgtg caggtggact acatccaggt gaacggcag
acgcgccagt ccgaggcaca gagctacaac accggcgcct acctgaacgg ccgttgcggc
                                                                                                                                                                    840
                                                                                                                                                                    900
                                                                                                                                                                    960
                                                                                                                                                                  1020
ggtggcggca acagcgaatg gatgcattgc aacggcgcca tcggctacgg caatacgccc
                                                                                                                                                                  1080
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tga

1083

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<211> 360
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(29)
<400> 218
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1 10 15
Leu Cys Leu Ala Gly Leu Tyr Met Ala Pro Ala Asn Ala Gln Thr Cys 20 25 30
The Thr Ser Ser Gln Thr Gly Thr Asn Asn Gly Asn Tyr Phe Ser Phe 35 40 45
Trp Lys Asp Ser Pro Gly Thr Val Asn Phe Cys Met Tyr Pro Asn Gly 50 55 60
Arg Tyr Thr Ser Asn Trp Ser Gly Ile Asn Asn Trp Val Gly Gly Lys
65 70 75 80
Gly Trp Ser Thr Gly Ser Ser Arg Thr Val Ser Tyr Ser Gly Ser Phe
Asn Ser Pro Gly Asn Gly Tyr Leu Thr Leu Tyr Gly Trp Thr Thr Asn 100 105 110
Pro Leu Ile Glu Tyr Tyr Ile Val Glu Asn Trp Gly Asn Tyr Arg Pro
115 120 125
         115
Pro Gly Gly Gln Gly Tyr Met Gly Thr Val Asn Ser Asp Gly Ala Thr
130 135 140
Tyr Asp Ile Tyr Arg Thr Phe Arg Asp Asn Gln Pro Cys Ile Thr Gly 155 160
Asn Ser Cys Asp Phe Tyr Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg
Ser Ser Gly Thr Ile Thr Thr Ala Asn His Phe Ala Ala Trp Asn Ser
180 185 190
Leu Gly Met Asn Leu Gly Gln His Asn Tyr Gln Val Met Ala Thr Glu
195 200 205
Gly Tyr Gln Ser Ser Gly Ser Ser Asp Ile Thr Val Thr Glu Gly Gly 210 220
Gly Gly Ser Ser Asn Gly Gly Ser Ser Asn Gly Gly Ser Ser Asn Gly 225 230 240
Gly Ser Ser Asn Gly Gly Gly Gly Gly Thr Lys Ser Phe Thr Val Arg
245 250 255
Ala Arg Gly Thr Ala Gly Gly Glu Ser Ile Thr Leu Arg Val Asn Asn 260 270
Gln Asn Val Gln Thr Trp Thr Leu Gly Thr Gly Met Gln Asn Tyr Thr
275 280 285
Ala Ser Thr Ser Leu Ser Gly Gly Ile Thr Val His Phe Thr Asn Asp 290 295 300
Gly Gly Ser Arg Asp Val Gln Val Asp Tyr Ile Gln Val Asn Gly Ser 305 310 315 320
Thr Arg Gln Ser Glu Ala Gln Ser Tyr Asn Thr Gly Ala Tyr Leu Asn 325
Gly Arg Cys Gly Gly Gly Asn Ser Glu Trp Met His Cys Asn Gly 340 345 350
Ala Ile Gly Tyr Gly Asn Thr Pro
355 360
<210> 219
<211> 1029
 <212> DNA
 <213> Unknown
 <223> Obtained from an environmental sample
 <400> 219
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gcgcatgccc agacatgtat tcagtccagt cagaccggca ccaacaacgg attctatttc
```

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60 120

```
tccttctgga aggacaaccc gggcacggtg cagttctgcc tgcagagcgg cggtcgttac
                                                                                      180
240
                                                                                      300
                                                                                      360
                                                                                      420
gacatctatc gcgcgcatcg cagtaacgcg ccctgcatca ccggcagcag ctgcgacttc
                                                                                      480
gaccagtact ggagcgtgcg acagtcgaag cgcgtcggcg gcaccatcac caccggcaac cacttcgatg cctgggcgaa ccaccagatg aatctgggcc agttcaacta ccagatcatg gctaccgagg gtttccagag caacggcagc tccgacatca ccgtcagtga atgcaccagc
                                                                                      540
                                                                                      600
                                                                                      660
aattgcggcg gtggcggcgg cggcgggggt ggcagcaaca gcatcacggt gcgcgcgcgc
                                                                                      720
ggcacgggcg gcggcgagca gatccggctg cgggtgaaca acaccacggt gcaaacctgg
                                                                                      780
acgctgacca ccagctacca gaacttcacg gcttcgacct cgctgagcgg cggcaccatc
gtcgagtact tcaacgacag ttccggccat gacgtgcagg tcgactacat catcgtgaat
ggcgtgaccc gccagtccga atcgcagagc tacaacaccg ggctgtatgc caacgggcgt
                                                                                      840
                                                                                      900
                                                                                     960
tgcggcggcg gctccaacag cgagtggatg cattgcaacg gtgccattgg atacggaaat
                                                                                     1020
accccgtaa
                                                                                     1029
<210> 220
<211> 342
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(23)
<400> 220
Met Thr Ser Gly Leu Lys Lys Val Met Ala Phe Val Cys Leu Ala Thr
1 5 10 15
Leu Gly Val Ser Ala His Ala Gln Thr Cys Ile Gln Ser Ser Gln Thr
Gly Thr Asn Asn Gly Phe Tyr Phe Ser Phe Trp Lys Asp Asn Pro Gly 35 40 45
Thr Val Gln Phe Cys Leu Gln Ser Gly Gly Arg Tyr Thr Ser Asn Trp 50 55 60
Asn Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Gln Thr Gly Ala 70 75 80
Arg Arg Thr Val Asn Tyr Ser Gly Ser Phe Asn Ser Pro Gly Asn Gly 85 90 95
Tyr Leu Ala Leu Tyr Gly Trp Thr Thr Asn Pro Leu Val Glu Tyr Tyr 100 105 110
Ile Val Asp Ser Trp Gly Ser Phe Arg Pro Pro Gly Asn Thr Ala Gly
115
120
125
Leu Trp Val Leu Val Asn Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Arg
130 135 140
Ala His Arg Ser Asn Ala Pro Cys Ile Thr Gly Ser Ser Cys Asp Phe
150 ______ 155 _____ 160
Asp Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Val Gly Gly Thr Ile
165 170 175
Thr Thr Gly Asn His Phe Asp Ala Trp Ala Asn His Gln Met Asn Leu
180 190
Gly Gln Phe Asn Tyr Gln Ile Met Ala Thr Glu Gly Phe Gln Ser Asn
195 200 205
Gly Ser Ser Asp Ile Thr Val Ser Glu Cys Thr Ser Asn Cys Gly Gly 210 220
Gly Gly Gly Gly Gly Gly Ser Asn Ser Ile Thr Val Arg Ala Arg 225 230 240
Gly Thr Gly Gly Glu Gln Ile Arg Leu Arg Val Asn Asn Thr Thr
245 250 255
Val Gln Thr Trp Thr Leu Thr Thr Ser Tyr Gln Asn Phe Thr Ala Ser
Thr Ser Leu Ser Gly Gly Thr Ile Val Glu Tyr Phe Asn Asp Ser Ser 275 280 285
Gly His Asp Val Gln Val Asp Tyr Ile Ile Val Asn Gly Val Thr Arg
290 295 300
Gln Ser Glu Ser Gln Ser Tyr Asn Thr Gly Leu Tyr Ala Asn Gly Arg
305 310 315 320
```

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Cys Gly Gly Gly Ser Asn Ser Glu Trp Met His Cys Asn Gly Ala Ile
                     325
                                               330
Gly Tyr Gly Asn Thr Pro
 <210> 221
 <211> 1044
 <212> DNA
 <213> Unknown
 <220>
 <223> Obtained from an environmental sample
 <400> 221
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 gccgcgcagg cgcaaacctg catcaattcc agccagaccg gcaccaacaa cggcaattat
                                                                                          120
ttttcattct ggaaagacaa cccgggcacg gtgaccttct gcatgtatgc caacggccgc tacacctcca actggagcgg catcaacaac tgggtgggtg gcaagggctg gcagaccggc tcgaatcgca cggtgaccta ctccggttcg ttcaactcgc ccggcaacgg ctacctcacc
                                                                                          180
                                                                                          240
                                                                                          300
ctgtacgggt ggaccacgaa tccgctgatc gagtactaca tcgtcgacag ttggggcagt
                                                                                          360
tatcgaccgc ccggcggcca gggcttcatg ggcaccgtga cgaccgacgg cggcacctac gacatctatc gcacgcagcg cgtgaaccag ccttccatca tcggcaccgc gacgttctac
                                                                                          420
                                                                                          480
cagtactgga gcgtgcggca gtcgaagcgc gtggggggga ccatcaccac cgccaaccac ttcaatgcct gggcgacgct gggcatgaac ctgggccagc acaactacca ggtcatggcc
                                                                                          540
                                                                                          600
accgagggtt accagagcag cggcagctcc gacatcaccg tgaccgaagg cggcggcagc
                                                                                          660
tcgtcgtcgt cgagcggcgg cggcagcacc agcagcggcg gtggcggcag caagagcttc
acggtgcgcg cccgcggcac ggtcggcggc gaaaacatcc agctgcaggt caacaaccag
acggtggcga gctggaacct gaccaccagc atgcagaact acaacgcctc gaccagcctg
                                                                                          720
                                                                                          780
                                                                                          840
agtggcggca tcaccgtggt ctacaccaac gacggcggta accgcgacgt ccaggtcgac
                                                                                         900
tacatcaccg tgaacggcca gacccgccag tccgaagcgc agagtttcaa caccgggctg
                                                                                         960
tatgccaacg gacgttgtgg cggcggctcg aacagcgagt ggatgcattg caatggcgcg
                                                                                        1020
atcggctacg gcaacacgcc gtaa
                                                                                        1044
<210> 222
<211> 347
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(24)
<400> 222
Met Ile Val Ser Phe Lys Ser Val Lys Ala Leu Ala Cys Leu Ala Val
                                              10
Leu Gly Val Thr Ala Ala Gln Ala Gln Thr Cys Ile Asn Ser Ser Gln
20 25 30
Thr Gly Thr Asn Asn Gly Asn Tyr Phe Ser Phe Trp Lys Asp Asn Pro
35 40 45
Gly Thr Val Thr Phe Cys Met Tyr Ala Asn Gly Arg Tyr Thr Ser Asn 50 60
Trp Ser Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Gln Thr Gly 65 75 80
Ser Asn Arg Thr Val Thr Tyr Ser Gly Ser Phe Asn Ser Pro Gly Asn 85 90 95
Gly Tyr Leu Thr Leu Tyr Gly Trp Thr Thr Asn Pro Leu Ile Glu Tyr 100 105 110
Tyr Ile Val Asp Ser Trp Gly Ser Tyr Arg Pro Pro Gly Gly Gln Gly 115 120 125
                                   120
Phe Met Gly Thr Val Thr Thr Asp Gly Gly Thr Tyr Asp Ile Tyr Arg
130 140
                              135
Thr Gln Arg Val Asn Gln Pro Ser Ile Ile Gly Thr Ala Thr Phe Tyr
                        150
Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Val Gly Gly Thr Ile Thr
165 170 175
Thr Ala Asn His Phe Asn Ala Trp Ala Thr Leu Gly Met Asn Leu Gly 180
```

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Gln His Asn Tyr Gln Val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly
          195
                                    200
                                                             205
Ser Ser Asp Ile Thr Val Thr Glu Gly Gly Gly Ser Ser Ser Ser Ser
     210
                              215
                                                       220
Ser Gly Gly Gly Ser Thr Ser Ser Gly Gly Gly Ser Lys Ser Phe 225 235 240
Thr Val Arg Ala Arg Gly Thr Val Gly Gly Glu Asn Ile Gln Leu Gln 245 250 255
Val Asn Asn Gln Thr Val Ala Ser Trp Asn Leu Thr Thr Ser Met Gln
               260
                                                                  270
                                        265
Asn Tyr Asn Ala Ser Thr Ser Leu Ser Gly Gly Ile Thr Val Val Tyr
          275
                                   280
Thr Asn Asp Gly Gly Asn Arg Asp Val Gln Val Asp Tyr Ile Thr Val
290 295 300
Ash Gly Gln Thr Arg Gln Ser Glu Ala Gln Ser Phe Ash Thr Gly Leu
305
                         310
                                                  315
                                                                            320
Tyr Ala Asn Gly Arg Cys Gly Gly Gly Ser Asn Ser Glu Trp Met His
Cys Asn Gly Ala Ile Gly Tyr Gly Asn Thr Pro
340 345
<210> 223
<211> 642
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample
<400> 223
atgittaagt ttaaaaagaa titcitagti ggattatcgg cagcittaat gagtattagc tigitticgg caaccgcctc tgcagctagc acagactact ggcaaaattg gactgatggg ggcggtatag taaacgctgt caatgggtct ggcgggaatt acagtgitaa tiggictaat
                                                                                         60
                                                                                        120
                                                                                        180
accggaaatt tcgttgttgg taaaggttgg actacaggtt cgccatttag gacgataaac tataatgccg gagtttgggc accgaatgga aatggatatt taactttata tggttggacg agatcaccct tcatagaata ttatgtagtg gattcatggg gactatatag acctactgga
                                                                                        240
                                                                                        300
                                                                                        360
acgtataaag gtactgtaaa aagtgatggg ggtacatatg acatatatac aactacacgt tataacgcac cttccattga tggcgatcgc actactttta cgcagtactg gagtgttcgc
                                                                                        420
                                                                                        480
caaacgaaga gaccaaccgg aagcaacgct acaatcactt tcagcaatca tgttaacgca
                                                                                        540
tggaagagcc atggaatgaa tctgggcagt aattgggctt accaagtcat ggcgacagaa
                                                                                        600
ggatatcaaa gtagtggaag ttctaacgta acagtgtggt aa
                                                                                        642
<210> 224
<211> 213
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(28)
<400> 224
Met Phe Lys Phe Lys Lys Asn Phe Leu Val Gly Leu Ser Ala Ala Leu
1 10 15
Met Ser Ile Ser Leu Phe Ser Ala Thr Ala Ser Ala Ala Ser Thr Asp
20 25 30
Tyr Trp Gln Asn Trp Thr Asp Gly Gly Gly Ile Val Asn Ala Val Asn 35 40 45
Gly Ser Gly Gly Asn Tyr Ser Val Asn Trp Ser Asn Thr Gly Asn Phe 50 60
Val Val Gly Lys Gly Trp Thr Thr Gly Ser Pro Phe Arg Thr Ile Asn 75 80
Tyr Asn Ala Gly Val Trp Ala Pro Asn Gly Asn Gly Tyr Leu Thr Leu
85 90 95
Tyr Gly Trp Thr Arg Ser Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser
Trp Gly Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Thr Val Lys Ser
```

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Asp Gly Gly Thr Tyr Asp Ile Tyr Thr Thr Thr Arg Tyr Asn Ala Pro
130 135 140
Ser Ile Asp Gly Asp Arg Thr Thr Phe Thr Gln Tyr Trp Ser Val Arg
145 150 155 160
Gin Thr Lys Arg Pro Thr Gly Ser Asn Ala Thr Ile Thr Phe Ser Asn
                                            170
                   165
His Val Asn Ala Trp Lys Ser His Gly Met Asn Leu Gly
180 185
                                                                190
Ala Tyr Gln val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser Ser
         195
Asn Val Thr Val Trp
     210
<210> 225
<211> 1059
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 225
                                                                                        60
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                                                                                       120
tcgacttcac aagcccagac ctgcatcacg tccagcggga cgggcaccaa caacggccac
                                                                                       180
tactattcct tctggaagga cagtggcggc accgtcaact tctgcatgta cgcgaacggc
cgctacacct ccaactggag cggcatcaac aactgggtgg gcggcaaggg ctggcagacc
ggctcacgcc ggacgatcag ctactcgggc tcgttcaact cacccggcaa tggttatctc
                                                                                       240
                                                                                       300
                                                                                       360
accetgtacg gitggaccae caatceatig ategagtact acategtega caactgggge
                                                                                       420
acgtaccggc cgccgggagg ctcgggctac atgggcacgg tgacgagcga cggcggcacc
tacgacgict atcgcaccca gcgcgtaaac cagccitcca tcatcggcac cgcgacgitc tatcaatact ggagcgigc ccagcagaag cggaccggcg ggaccatcac caccggcaat
                                                                                       480
                                                                                       540
                                                                                       600
cacttcgacg cctgggccgc atacggaatg aacctcggca cccacaacta ccagatcatg
                                                                                       660
gcgaccgagg gttaccagag cagcggcagt tcggacatca cggtgagcga gggcggtggc
agttcatcga gcagcagctc gtcgagcagc agcagttcgt cctcttcgag cggcggcggc
ggcacgaaga gcttcacggt ccgcgcgcg ggcacggcgg gcggtgaatc catcacgctg
cgcgtgaaca accagaacgt gcagacctgg acgctgggca cgtcgatgca gaactacacc
gcatcgacca cgctctccgg tggcatcacc gtcgcgtaca ccacacacac cgcaatcga
                                                                                       720
                                                                                       780
                                                                                       840
                                                                                       900
                                                                                       960
gacgtgcagg tggactacat cgtcgtgaac ggcgccaccc gccagtccga ggcgcagagc
                                                                                      1020
tacāacaccg gtctctatgc caacggtcgt tgcggcggcg gctccaacag cgagtggatg
                                                                                      1059
cactgcaacg ggcagatcgg ctacgggaat actccctag
<210> 226
<211> 352
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(25)
<400> 226
Met Phe Val Ser Leu Arg Lys Thr Ala Trp Ala Cys Leu Leu Leu Ala
Gly Leu Gly Ile Ser Thr Ser Gln Ala Gln Thr Cys Ile Thr Ser Ser 20 25 30
Gly Thr Gly Thr Asn Asn Gly His Tyr Tyr Ser Phe Trp Lys Asp Ser 40 45
Gly Gly Thr Val Asn Phe Cys Met Tyr Ala Asn Gly Arg Tyr Thr Ser
Asn Trp Ser Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Gln Thr
65 70 75 80
Gly Ser Arg Arg Thr Ile Ser Tyr Ser Gly Ser Phe Asn Ser Pro Gly
85 90 95
Asn Gly Tyr Leu Thr Leu Tyr Gly Trp Thr Thr Asn Pro Leu Ile Glu
100 105 110
```

Tyr Tyr Ile Val Asp Asn Trp Gly Thr Tyr Arg Pro Pro Gly Gly Ser

```
Gly Tyr Met Gly Thr Val Thr Ser Asp Gly Gly Thr Tyr Asp Val Tyr
                                                        140
                              135
Arg Thr Gln Arg Val Asn Gln Pro Ser Ile Ile Gly Thr Ala Thr Phe 145 150 155 160
Tyr Gln Tyr Trp Ser Val Arg Gln Gln Lys Arg Thr Gly Gly Thr Ile
165 170 175
Thr Thr Gly Asn His Phe Asp Ala Trp Ala Ala Tyr Gly Met Asn Leu
180 185 190
Gly Thr His Asn Tyr Gln Ile Met Ala Thr Glu Gly Tyr Gln Ser Ser
195 200 205
Gly Ser Ser Asp Ile Thr Val Ser Glu Gly Gly Gly Ser Ser Ser Ser 210 220
Gly Thr Lys Ser Phe Thr Val Arg Ala Arg Gly Thr Ala Gly Gly Glu 255
Ser Ile Thr Leu Arg Val Asn Asn Gln Asn Val Gln Thr Trp Thr Leu 260 265 270
Gly Thr Ser Met Gln Asn Tyr Thr Ala Ser Thr Thr Leu Ser Gly Gly 285
Ile Thr Val Ala Tyr Thr Asn Asp Ser Gly Asn Arg Asp Val Gln Val
Asp Tyr Ile Val Val Asn Gly Ala Thr Arg Gln Ser Glu Ala Gln Ser
305 310 315 320
Tyr Asn Thr Gly Leu Tyr Ala Asn Gly Arg Cys Gly Gly Ser Asn 325
Ser Glu Trp Met His Cys Asn Gly Gln Ile Gly Tyr Gly Asn Thr Pro
<210> 227
<211> 747
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 227
                                                                                            60
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gcgggcggta ccgccggagc gctcggcccc ggcggtaccc agggcagcgg tggcgcagcc
ggtggtacga gcggaacggg cggggccatc agcagcagct gcacggaagc tgacaagacg
                                                                                           120
                                                                                           180
gtctgcaaca acgaaaccgg tcgccactgc aattacacgt acgagtattg gaaggaccag
                                                                                           240
                                                                                           300
ggaagcggtt gcctcgtgaa caaagccgac ggcttcagcg tcaactggaa caacatcaac
aatctgctgg gtcgcaaggg tctgaggccc ggatcgtcga atcagacggt gacctaccag gcaaactacc agccgaacgg caattcatac ctgtgcgta atggatggac gcaaaacccc ctcgtcgaat actacatcgt cgatagctgg ggcagctggc gcccgccggg gggaacgtcc atgggcaccg tcaacgcgga cggcggcacc tacgacatct accgcaccca gcgcgtcaac
                                                                                           360
                                                                                           420
                                                                                           480
                                                                                           540
cagcetteca tegaaggeac caagacette tateaatact ggagegtteg caetcagaag egeacgageg gaacgateac ggttgeeget caettegaeg eetgggegae gaaggggatg
                                                                                           600
                                                                                           660
                                                                                           720
 aācatgggga gtctgtacga ggtgtcgatg accgtcgagg gctatcaaag cagcgggacc
                                                                                           747
 gccgacgtga gcttctcgat gaagtga
 <210> 228
<211> 248
 <212> PRT
 <213> Unknown
 <220>
 <223> Obtained from an environmental sample
 <221> SIGNAL
 <222> (1)...(39)
 <400> 228
Met Gly Gly Thr Thr Gly Ser Gly Gly Ser Ala Ala Ala Gly Ala Gly 10 15 15 10 15 Thr Ser Gly Ser Ala Gly Gly Thr Ala Gly Ala Leu Gly Pro Gly Gly 20 25 30
```

```
Thr Gln Gly Ser Gly Gly Ala Ala Gly Gly Thr Ser Gly Thr Gly Gly
                                       40
 Ala Ile Ser Ser Ser Cys Thr Glu Ala Asp Lys Thr Val Cys Asn Asn 50 55 60
 Glu Thr Gly Arg His Cys Asn Tyr Thr Tyr Glu Tyr Trp Lys Asp Gln 65 70 75 80
 Gly Ser Gly Cys Leu Val Asn Lys Ala Asp Gly Phe Ser Val Asn Trp
85 90 _ 95
 Asn Asn Ile Asn Asn Leu Leu Gly Arg Lys Gly Leu Arg Pro Gly Ser
 Ser Asn Gln Thr Val Thr Tyr Gln Ala Asn Tyr Gln Pro Asn Gly Asn
115 120 125
 Ser Tyr Leu Cys Val Tyr Gly Trp Thr Gln Asn Pro Leu Val Glu Tyr
130 140
 Tyr Ile Val Asp Ser Trp Gly Ser Trp Arg Pro Pro Gly Gly Thr Ser
145 150 155 160
Met Gly Thr Val Asn Ala Asp Gly Gly Thr Tyr Asp Ile Tyr Arg Thr
165 170 175
Gln Arg Val Asn Gln Pro Ser Ile Glu Gly Thr Lys Thr Phe Tyr Gln
                                            185
Tyr Trp Ser Val Arg Thr Gln Lys Arg Thr Ser Gly Thr Ile Thr Val
Ala Ala His Phe Asp Ala Trp Ala Thr Lys Gly Met Asn Met Gly Ser 210 220
Leu Tyr Glu Val Ser Met Thr Val Glu Gly Tyr Gln Ser Ser Gly Thr
225 230 235 240
Ala Asp Val Ser Phe Ser Met Lys
<210> 229
<211> 642
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample
<400> 229
atgtttaagt ttacaaagaa attcttagtt gggttaacgg cagctttgat gagtattagc ttgttttcgg caaacgcctc tgcagctaac acagactact ggcaaaattg gactgatggg ggcggaacag taaacgctgt caatgggtcg ggcgggaatt acagtgtgaa ttggtctaat accgggaatt tcgttgttgg taaaggttgg actacaggt cgccattag gacgataaac
                                                                                                  60
                                                                                                 120
                                                                                                 180
                                                                                                 240
tataatgccg gagtttgggc gccgaatggc aatgcatatt tgactttata tggttggacg cgatcacccc tcatagaata ttatgtagtg gattcatggg gtacttatag acctactgga acgtataaag gtacggttta cagtgatggg ggtacatatg acgtgtacac aactacacgt tatgatgcac cttccattga tggcgataaa actactttta cgcagtactg gagtgttcgc cagtcgaaga gaccaactgg aagcaacgct acaatcatt tcagcaatca cgttaacgca
                                                                                                 300
                                                                                                 360
                                                                                                 420
                                                                                                 480
                                                                                                 540
tggaagagat atgggatgaa tctgggtagt aattggtctt accaagtctt agcgacagag
                                                                                                 600
ggatatcaaa gtagtggaag ttctaacgta acagtgtggt aa
                                                                                                 642
<210> 230
<211> 213
<212> PRT
<213> Unknown
<223> Obtained from an environmental sample
<221> SIGNAL
<222> (1)...(28)
<400> 230
Met Phe Lys Phe Thr Lys Lys Phe Leu Val Gly Leu Thr Ala Ala Leu
1 15
Met Ser Ile Ser Leu Phe Ser Ala Asn Ala Ser Ala Ala Asn Thr Asp
Tyr Trp Gln Asn Trp Thr Asp Gly Gly Gly Thr Val Asn Ala Val Asn 45
Gly Ser Gly Gly Asn Tyr Ser Val Asn Trp Ser Asn Thr Gly Asn Phe
```

```
Val Val Gly Lys Gly Trp Thr Thr Gly Ser Pro Phe Arg Thr Ile Asn 70 75 80
 Tyr Asn Ala Gly Val Trp Ala Pro Asn Gly Asn Ala Tyr Leu Thr Leu
85 90 95
 Tyr Gly Trp Thr Arg Ser Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser
100 105 110
 Trp Gly Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Thr Val Tyr Ser
 Asp Gly Gly Thr Tyr Asp Val Tyr Thr Thr Arg Tyr Asp Ala Pro
130
135
140
 Ser Ile Asp Gly Asp Lys Thr Thr Phe Thr Gln Tyr Trp Ser Val Arg
145 155 160
 Gln Ser Lys Arg Pro Thr Gly Ser Asn Ala Thr Ile Thr Phe Ser Asn
165 170 175
His Val Asn Ala Trp Lys Arg Tyr Gly Met Asn Leu Gly Ser Asn Trp
 Ser Tyr Gln Val Leu Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser Ser
195 200 205
 Asn Val Thr Val Trp
       210
 <210> 231
 <211> 1008
 <212> DNA
 <213> Bacteria
 <400> 231
 atgaacctgc tcgtccagcc gaggcgtcgc agacgcggtc cggtcacctt gctcgtcagg
                                                                                                             60
agcgcgtggg ccgtcgcgct ggcggcgctc gccgcgctga tgctgccggg caccgccag
gccgacacgg tcgtcacgac caaccaggag ggcaccaaca acggctacta ctactcgttc
tggaccgaca gccagggcac cgtctccatg aacatgggct ccggcggtca gtacagcacc
                                                                                                           120
                                                                                                           180
                                                                                                           240
tcgtggcgca acaccggcaa cttcgtcgcg ggcaagggct gggccaacgg cggccgcgg accgtgcagt actcgggcag cttcaaccc tccggcaacg cgtactggc gctctacgga tggacgtcga acccgctcgt cgagtactac atcgtcgaca actggggcac ctaccggcca acgggcgagt acaagggcac cgtcaccagc gacggcggca cctacgacat ctacaagacg acccgcgtca acaaggcctc cgtcgagggc acccgcacct tcgaccagta ctggagggtc
                                                                                                           300
                                                                                                           360
                                                                                                           420
                                                                                                           480
                                                                                                           540
 cggcaggcga agcggaccgg cggcaccatc acgaccggca accacttcga cgcgtgggcc
                                                                                                           600
Cgggccggga tgccgctcgg caacttcagc tactacatga tcatggccac cgagggctac cagaggcagcag gcagctccag catcaacgtc ggcgggaccg gccgcggcga caacggcggc ggcgaccagg gggggacggg cggcggggtgc accgccacgg tgtccgccgg gcagaagtgg ggcgaccggt acaacctcga cgtctccgtc agcggcgac gcgaccggac ggtgacgatg
                                                                                                           660
                                                                                                           720
                                                                                                           780
                                                                                                           840
aacgtgccgt ccccggcgaa ggtcctgtcg acctggaacg tcaacgccag ctatcccagt gcgcagacgc tgaccgccag gtcgaacggc agcggcaaca actggggcgc caccatccag gccaacggca actggacctg gcccagcgtg tcctgcagcg cgggctga
                                                                                                           900
                                                                                                           960
                                                                                                         1008
<210> 232
<211> 335
<212> PRT
<213> Bacteria
<220>
<221> SIGNAL
<222> (1)...(41)
<400> 232
Met Asn Leu Leu Val Gln Pro Arg Arg Arg Arg Gly Pro Val Thr
1 5 10 15
Leu Leu Val Arg Ser Ala Trp Ala Val Ala Leu Ala Ala Leu Ala Ala 20 25 30
Leu Met Leu Pro Gly Thr Ala Gln Ala Asp Thr Val Val Thr Thr Asn
45
Gln Glu Gly Thr Asn Asn Gly Tyr Tyr Tyr Ser Phe Trp Thr Asp Ser
Gln Gly Thr Val Ser Met Asn Met Gly Ser Gly Gly Gln Tyr Ser Thr
65 70 75 80
Ser Trp Arg Asn Thr Gly Asn Phe Val Ala Gly Lys Gly Trp Ala Asn 90 95
Gly Gly Arg Arg Thr Val Gln Tyr Ser Gly Ser Phe Asn Pro Ser Gly
```

```
Asn Ala Tyr Leu Ala Leu Tyr Gly Trp Thr Ser Asn Pro Leu Val Glu 115
Tyr Tyr Ile Val Asp Asn Trp Gly Thr Tyr Arg Pro Thr Gly Glu Tyr 130 135 140
Lys Gly Thr Val Thr Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Lys Thr
145 150 155 160
Thr Arg Val Asn Lys Pro Ser Val Glu Gly Thr Arg Thr Phe Asp Gln
165 170 175
Tyr Trp Ser Val Arg Gln Ala Lys Arg Thr Gly Gly Thr Ile Thr Thr 180 185 190
Gly Asn His Phe Asp Ala Trp Ala Arg Ala Gly Met Pro Leu Gly Asn
195 200 205
Phe Ser Tyr Tyr Met Ile Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly 210 220
Ser Ser Ser Ile Asn Val Gly Gly Thr Gly Arg Gly Asp Asn Gly Gly 235 240
Gly Asp Asn Gly Gly Gly Gly Gly Cys Thr Ala Thr Val Ser Ala 245 250 255
Gly Gln Lys Trp Gly Asp Arg Tyr Asn Leu Asp Val Ser Val Ser Gly 260 265 270
Ala Ser Asp Trp Thr Val Thr Met Asn Val Pro Ser Pro Ala Lys Val
275 280 285
Leu Ser Thr Trp Asn Val Asn Ala Ser Tyr Pro Ser Ala Gln Thr Leu 290 295 300
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acctctatg ggtggaccac caatccgctc atcgagtact acatcgtcga caactggggc
tcgtatcgcc cgccgggcgg acaggggttc atgggcacgg tgaccagcga cggcggcacg
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Gln Thr Gly Thr Asn Asn Gly Phe Tyr Phe Ser Phe Trp Lys Asp Ser
Pro Gly Thr Val Asn Phe Cys Asn Gln Ser Gly Gly Arg Tyr Thr Ser 50 55
Asn Trp Ser Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Gln Thr 65 70 75 80
Gly Ser Arg Arg Val Val Ser Tyr Ser Gly Ser Phe Asn Ser Pro Gly
85 90 95
Asn Gly Tyr Leu Thr Leu Tyr Gly Trp Thr Thr Asn Pro Leu Ile Glu 100 110
Tyr Tyr Ile Val Asp Asn Trp Gly Ser Tyr Arg Pro Pro Gly Gly Gln
115 120 125
Gly Phe Met Gly Thr Val Thr Ser Asp Gly Gly Thr Tyr Asp Val Tyr
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135
140
Arg Thr Gln Arg Val Asn Gln Pro Cys Ile Thr Gly Ser Ser Cys Thr
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Phe Tyr Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Thr Gly Gly Thr
165 170 175
lle Thr Thr Gly Asn His Phe Asp Ala Trp Ala Ser Tyr Gly Met Asn 180 185
Leu Gly Ala His Asn Tyr Gln Ile Met Ala Thr Glu Gly Tyr Gln Ser
Ser Gly Ser Ser Asp Ile Thr Val Ser Glu Gly Ser Ser Ser Ser Ser 210 220
Ser Gly Gly Gly Thr Lys Ser Phe Thr Val Arg Ala Arg Gly Val
245 250 255
Ala Gly Gly Glu Ser Ile Thr Leu Arg Val Asn Asn Gln Asn Val Gln
260 265 270
Thr Trp Thr Leu Gly Thr Gly Met Gln Asn Tyr Thr Ala Ser Thr Ser 285
Leu Ser Gly Gly Ile Thr Val Ala Tyr Thr Asn Asp Gly Gly Ser Arg
Asp Val Gln Val Asp Tyr Ile Ile Val Asn Gly Gln Thr Arg Gln Ser 305 310 315 320
Glu Ala Gln Ser Tyr Asn Thr Gly Leu Tyr Ala Asn Gly Arg Cys Gly
325 330 335
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Gly Asn Thr Pro
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gtcaattatc tgaataccca gggctcgcgt tttggtgatc tgaaagtgat tgcgccggaa
tccctgggtt tcacgacctc gtattccgac cccatcctca acagcgccac ggcagcgccg
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720

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Asn Ala Pro Gly Trp Ile Asp Asp Leu Thr Thr Ala Gln Val Asn Lys
50
60
Ala Tyr Gly Ser Gly Asp Gly Gln Val Gly Leu Ser Ile Met Arg Met 65 70 75 80
Arg Ile Asp Pro Asn Ser Ala Ala Trp Asn Ile Gln Val Pro Ala Ala
85 _ _ _ 90 _ _ 95
Lys Arg Ala Lys Glu Leu Gly Ala Ile Leu Phe Ala Thr Pro Trp Ser
Pro Pro Ala Tyr Met Lys Ser Asn Lys Ser Leu Asn Asn Gly Gly Lys
115 120 125
Leu Leu Pro Glu Tyr Tyr Ser Ala Tyr Thr Thr His Leu Leu Asp Phe
130
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Ala Ser Phe Met Ser Arg Asn Gly Ala Pro Leu Tyr Ala Ile Ser Ile
145 150 160
Gln Asn Glu Pro Asp Trp Leu Pro Asp Tyr Glu Ser Cys Ala Trp Thr
165 170 175
Gly Thr Asp Phe Val Asn Tyr Leu Asn Thr Gln Gly Ser Arg Phe Gly
180
185
190
Asp Leu Lys Val Ile Ala Pro Glu Ser Leu Gly Phe Thr Thr Ser Tyr
Ser Asp Pro Ile Leu Asn Ser Ala Thr Ala Ala Pro His Val Asp Ile 210 220
Ile Gly Gly His Leu Tyr Gly Val Leu Pro Lys Asp Tyr Pro Leu Ala
225 230 240
Arg Gln Lys Gly Lys Glu Ile Trp Met Thr Glu His Tyr Thr Glu Ser
245 250
Lys Asn Ser Gly Asp Ala Trp Pro Leu Ala Leu Asp Val Gly Thr Glu 260 265 270
Leu His Gln Ser Met Val Ala Asn Tyr Asn Ala Tyr Val Trp Trp Tyr 285
Val Arg Arg Ser Tyr Gly Leu Leu Leu Glu Asn Gly Asn Val Ser Lys
290 295 300
Arg Gly Tyr Ile Met Ser Gln Tyr Ala Arg Phe Val Arg Pro Gly Ser 315 320
Lys Arg Ile Gly Ala Thr Glu Lys Pro His Ala Asp Val Ala Val Thr
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Ala Tyr Lys Thr Pro Asp Asn Arg Ile Val Leu Val Ala Val Asn Thr
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 Gly Ser Phe Ser Lys Phe Ser Thr Ser Gly Thr Leu Asn Val Gly Ser
                                    375
                                                                   380
 Gly Gly Ser Tyr Lys Val Asn Asn Gly Ala Val Ser Leu Tyr Ile Asp
385 390 395
                               390
                                                             395
 Pro Gln Ser Val Ala Thr Leu Val Gly Asp Leu Pro Gly Thr Ala Ser
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 Ser Ser Ser Ala Ser Gly Ala Pro Ala Leu Ser Gly Ser Ser Asp Tyr
435 440 445
Pro Thr Gly Phe Ser Lys Cys Ala Asp Leu Gly Gly Thr Cys Ala Val
Pro Ser Gly Ser Gly Trp Thr Ala Phe Gly Arg Lys Gly Lys Trp Val
465 470 480
Ala Lys Tyr Val Gly Val Gly Lys Ser Ile Ala Cys Thr Val Thr Ala 485
Phe Gly Ser Asp Pro Gly Gly Ala Pro Asn Lys Cys Ser Tyr Gln Lys 500

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                                                                                                         900
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                                                                                                        1080
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1 5 10 15
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 Ala Ser Asp Val Thr Val Asn Val Ser Ala Glu Lys Gln Val Ile Arg
 Gly Phe Gly Gly Met Asn His Pro Ala Trp Ala Gly Asp Leu Thr Ala 50 55 60
 Ala Gln Arg Glu Thr Ala Phe Gly Asn Gly Gln Asn Gln Leu Gly Phe 65 70 75 80
 Ser Ile Leu Arg Ile His Val Asp Glu Asn Arg Asn Asn Trp Tyr Lys
 Glu Val Glu Thr Ala Lys Ser Ala Val Lys His Gly Ala Ile Val Phe
100 105 110
Ala Ser Pro Trp Asn Pro Pro Ser Asp Met Val Glu Thr Phe Asn Arg
115 120 125
Ash Gly Asp Thr Ser Ala Lys Arg Leu Lys Tyr Ash Lys Tyr Ala Ala
130 135 140
Tyr Ala Gln His Leu Asn Asp Phe Val Thr Phe Met Lys Asn Asn Gly 155 150 160
Val Asn Leu Tyr Ala Ile Ser Val Gln Asn Glu Pro Asp Tyr Ala His
165 170 175
Glu Trp Thr Trp Trp Thr Pro Gln Glu Ile Leu Arg Phe Met Arg Glu 180 185 190
Asn Ala Gly Ser Ile Asn Ala Arg Val Ile Ala Pro Glu Ser Phe Gln
195 200 205
Tyr Leu Lys Asn Leu Ser Asp Pro Ile Leu Asn Asp Pro Gln Ala Leu 210 220
Ala Asn Met Asp Ile Leu Gly Thr His Leu Tyr Gly Thr Gln Val Ser
235 230 235 240
Gln Phe Pro Tyr Pro Leu Phe Lys Gln Lys Gly Ala Gly Lys Asp Leu
245 250 255
Trp Met Thr Glu Val Tyr Tyr Pro Asn Ser Asp Thr Asn Ser Ala Asp 260 265 270
Arg Trp Pro Glu Ala Leu Asp Val Ser Gln His Ile His Asn Ala Met 275 280 285
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Val Glu Gly Asp Phe Gln Ala Tyr Val Trp Trp Tyr Ile Arg Arg Ser
290 _____ 295 _____ 300
Tyr Gly Pro Met Lys Glu Asp Gly Thr Ile Ser Lys Arg Gly Tyr Asn 305 310 320
Met Ala His Phe Ser Lys Phe Val Arg Pro Gly Tyr Val Arg Ile Asp 325 330 335
Ala Thr Lys Asn Pro Asn Ala Asn Val Tyr Val Ser Ala Tyr Lys Gly
Asp Asn Lys Val Val Ile Val Ala Ile Asn Lys Ser Asn Thr Gly Val
Asn Gln Asn Phe Val Leu Gln Asn Gly Ser Ala Ser Asn Val Ser Arg
370 380
Trp Ile Thr Ser Ser Ser Ser Asn Leu Gln Pro Gly Thr Asn Leu Thr 385 390 395 400
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atcggagatt tgacggcagc acagagagaa accgcattg ggaacgggcc aaatcagtta
ggcttctcga tattaagaat ctacgtgcat gaagaccgaa atcagtgca ccgtgaactg
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accgaggtgt attacccgaa cagcgacaac aactcggcgg atcgctggcc cgaagccctg
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Ala Ala Ser Asp Ala Val Ile Asn Val Ser Ser Glu Lys Gln Val
Ile Arg Gly Phe Gly Gly Ile Asn His Pro Ala Trp Ile Gly Asp Leu 50 60
Thr Ala Ala Gln Arg Glu Thr Ala Phe Gly Asn Gly Pro Asn Gln Leu
65 70 75 80
Gly Phe Ser Ile Leu Arg Ile Tyr Val His Glu Asp Arg Asn Gln Trp
His Arg Glu Leu Asp Thr Ala Lys Arg Ala Ile Ala Leu Gly Ala Ile
Val Phe Ala Ser Pro Trp Asn Pro Pro Ala Asp Met Val Glu Thr Phe
115 120 125
Asn Arg Asn Gly Asp Thr Ser Ala Lys Arg Leu Arg Tyr Asp Lys Tyr
130
140
Thr Ala Tyr Ala Gln His Leu Asn Asp Phe Val Thr Tyr Met Arg Asn 145 150 155 160
Asn Gly Val Asn Leu Tyr Ala Ile Ser Val Gln Asn Glu Pro Asp Tyr
165 170 175
Ala His Asp Trp Thr Trp Trp Thr Pro Gln Glu Met Leu Arg Phe Met
                                     185
Lys Glu Asn Ala Gly Ser Ile Asn Ser Arg Val Ile Ala Pro Glu Ser
195 200 205
Phe Gln Tyr Leu Lys Asn Met Ser Asp Pro Ile Leu Asn Asp Pro Gln 210 220
Ala Leu Ala Asn Met Asp Ile Leu Gly Ala His Leu Tyr Gly Thr Gln
225 230 240
Val Ser Asn Phe Ala Tyr Pro Leu Phe Lys Gln Lys Gly Ala Gly Lys
245
250
255
Asp Leu Trp Met Thr Glu Val Tyr Tyr Pro Asn Ser Asp Asn Asn Ser 260 265 270
Ala Asp Arg Trp Pro Glu Ala Leu Asp Val Ser Tyr His Ile His Asn 275 280 285
Ala Met Val Glu Gly Asp Phe Gln Ala Tyr Val Trp Trp Tyr Ile Arg
290 295 300
Arg Ser Tyr Gly Pro Met Lys Glu Asp Gly Thr Ile Ser Lys Arg Gly 305
Tyr Asn Met Ala His Phe Ser Lys Phe Val Arg Pro Gly Tyr Val Arg
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Val Asp Ala Ser Lys Asn Pro Glu Thr Asn Val Tyr Val Ser Ala Tyr
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Gly Val Asn Gln Asn Phe Val Leu Gln Asn Gly Ser Val Ser Gln Val
                                    375
Ser Arg Trp Ile Thr Ser Ser Ser Ser Asn Leu Gln Pro Gly Thr Ser
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                              390
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Val Thr Thr Phe Val Gly Glu Leu Gly Arg
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<211> 564
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 Leu Leu Ala Cys Leu Thr Ala Leu Pro Leu Met Leu Thr Pro Thr His 20 25 30
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35 40 45 Leu Ile Lys Gly Phe Gly Gly Ile Asn His Pro Ala Trp Ile Gly Asp 50 _____ 60 Leu Thr Ala Ala Gln Arg Glu Thr Ala Phe Gly Asn Gly Ala Asn Gln 80 Leu Gly Phe Ser Ile Leu Arg Ile Tyr Val Asp Glu Asn Pro Asn Asn 90 95 Trp Tyr Arg Glu Val Ala Thr Ala Lys Arg Ala Ile Glu Gln Gly Ala
100 105 110 Ile Val Phe Ala Ser Pro Trp Asn Pro Pro Ser Asp Met Val Glu Thr 115 120 125 Phe Asn Arg Asn Gly Asp Thr Asn Ala Lys Arg Leu Arg Tyr Asp Lys 130 140 Tyr Ala Ala Tyr Ala Gln His Leu Asn Asp Phe Val Ser Tyr Met Lys
145 150 155 160 Asn Asn Gly Val Asp Leu Tyr Ala Ile Ser Val Gln Asn Glu Pro Asp 165 170 175 Tyr Ala His Glu Trp Thr Trp Trp Thr Pro Gln Glu Ile Leu Arg Phe 180 190 Met Lys Glu Asn Ala Gly Ser Ile Gln Asn Thr Lys Val Met Ala Pro 195 200 205 Glu Ser Phe Gln Tyr Leu Lys Asn Met Ser Asp Pro Ile Leu Asn Asp 210 220 Pro Gln Ala Leu Ala Asn Met Asp Ile Leu Gly Ala His Thr Tyr Gly 235 230 240 Thr Gln Phe Lys Asp Phe Ala Tyr Pro Leu Phe Lys Gln Lys Gly Ala 245 250 255 Gly Lys Glu Leu Trp Met Thr Glu Val Tyr Tyr Pro Asn Ser Asp Asn 260 _____ 270 Asn Ser Ser Asp Arg Trp Pro Glu Ala Leu Asp Val Ser Tyr His Met 275 His Asn Ala Met Val Glu Gly Asp Phe Gln Ala Tyr Val Trp Trp Tyr 290 295 300 Ile Arg Arg Gln Tyr Gly Pro Met Asn Glu Asn Gly Thr Ile Ser Lys 315 320 Arg Gly Tyr Asn Met Ala His Phe Ser Lys Phe Val Arg Pro Gly Tyr 325 330 335 Tyr Arg Val Asp Ala Thr Lys Asn Pro Asp Thr Asn Thr Phe Val Ser Ala Tyr Lys Gly Asp Asn Lys Ala Val Ile Val Ala Ile Asn Arg Gly 355 Thr Ser Ala Val Ser Gln Lys Phe Val Leu Gln Asn Gly Asn Ala Ser 370 380 Thr Val Ser Ser Trp Val Thr Asp Ser Ser Arg Asn Leu Ala Ser Gly 385 390 395 Ala Pro Ile Thr Met Ser Gly Gly Ala Phe Thr Ala Gln Leu Pro Ala 405 410 415 Gln Ser Val Thr Thr Phe Val Ala Asn Ile Thr Gly Gly Ser Val Thr 420 430 Pro Gly Ser Gly Thr Thr Tyr Glu Ala Glu Thr Gly Thr Thr Leu Thr 435 440 445 Asp Ala Val Ile Glu Thr Leu Tyr Pro Gly Tyr Thr Gly Thr Gly Tyr
450 460 Val Asn Phe Asn Ala Tyr Thr Gly Ser Ala Ile Gln Trp Asn Ala Ile 465 470 480 475 Asn Asn Thr Ile Thr Gly Thr Lys Asn Val Lys Phe Arg Tyr Ala Gln 485 490 495 Glu Ser Gly Thr Arg Asn Leu Asp Ile Phe Val Asn Gly Thr Lys Val The Ser Asn Glu Pro Phe Pro Ala Thr Gly Ser Trp Ser Thr Trp Ser Glu Lys Thr Ile Gln Val Pro Met Asn Ala Gly Thr Asn Thr Ile Lys
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                                                                                                  120
                                                                                                  180
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ggagatītgā cagcagctca āagagāaācc gctītītggca atggacagaa īcagtīaggt
                                                                                                  240
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                                                                                                  300
                                                                                                  360
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gataagtacg ccgcatacgc gcagcatctt aacgattttg ttacctacat gaagaataat ggcgtgaatc tttatgcgat ttctgttcaa aacgagcctg attatgcgca cgaatggacg tggtggactc cgcaagaaat acttcgttc atgagagaaa atgccggttc cattaatgca cgtgtcattg caccagaatc ttttcagtac tttaaaaaata tatcggaccc cattagaac
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                                                                                                1140
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                                                                                                1200
aatgtaacgg gcaatcattt ttgggcccat cttccagctc aaagcgtgac aacatttgtc
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Ala Ala Ser Asp Val Thr Ile Asn Leu Ser Ala Glu Lys Gln Val Ile
Arg Gly Phe Gly Gly Met Asn His Pro Ala Trp Ile Gly Asp Leu Thr
Ala Ala Gln Arg Glu Thr Ala Phe Gly Asn Gly Gln Asn Gln Leu Gly
65 70 _ ___ 75 ____ 80
Phe Ser Ile Leu Arg Ile His Val Asp Glu Asn Arg Asn Asn Trp Tyr
85 _ 90 _ _ 95
Arg Glu Val Glu Thr Ala Lys Ser Ala Ile Lys His Gly Ala Ile Val
Phe Ala Ser Pro Trp Asn Pro Pro Ser Asp Met Val Glu Thr Phe Asn 115 120 125
Arg Asn Gly Asp Thr Ser Ala Lys Arg Leu Arg Tyr Asp Lys Tyr Ala 130 135 140
Ala Tyr Ala Gln His Leu Asn Asp Phe Val Thr Tyr Met Lys Asn Asn 145 150 160
Gly Val Asn Leu Tyr Ala Ile Ser Val Gln Asn Glu Pro Asp Tyr Ala
165 170 175
His Glu Trp Thr Trp Trp Thr Pro Gln Glu Ile Leu Arg Phe Met Arg
 Glu Asn Ala Gly Ser Ile Asn Ala Arg Val Ile Ala Pro Glu Ser Phe
                                                    Page 177
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Gln Tyr Phe Lys Asn Ile Ser Asp Pro Ile Leu Asn Asp Pro Gln Ala 210 220
 Leu Arg Asn Met Asp Ile Leu Gly Thr His Leu Tyr Gly Thr Gln Val
225 230 235 240
 Ser Gln Phe Pro Tyr Pro Leu Phe Lys Gln Lys Gly Ala Gly Lys Glu 245 250 255
 Leu Trp Met Thr Glu Val Tyr Tyr Pro Asn Ser Asp Asn Ser Ala 260 265 270
 Asp Arg Trp Pro Glu Ala Leu Gly Val Ser Glu His Ile His His Ser
275 280 285
 Met Val Glu Gly Asp Phe Gln Ser Tyr Val Trp Trp Tyr Ile Arg Arg
290 295 300
 Ser Tyr Gly Pro Met Lys Glu Asp Gly Thr Ile Ser Lys Arg Gly Tyr 305 310 315 320
 Asn Met Ala His Phe Ser Lys Phe Val Arg Pro Gly Tyr Val Arg Val 325 335
 Asp Ala Thr Lys Asn Pro Asn Ala Asn Val Tyr Val Ser Ala Tyr Lys 340 350
 Gly Asp Asn Lys Val Val Ile Val Ala Ile Asn Lys Ser Asn Thr Gly
Val Asn Gln Asn Phe Val Leu Gln Asn Gly Ser Ala Ser Gln Val Ser 370 380
Arg Trp Ile Thr Ser Gly Ser Ser Asn Leu Gln Pro Gly Thr Asn Leu 385 390 395
Asn Val Thr Gly Asn His Phe Trp Ala His Leu Pro Ala Gln Ser Val
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                                                          410
 Thr Thr Phe Val Ala Asn Arg
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 <211> 1263
 <212> DNA
 <213> Unknown
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                                                                                                                240
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                                                                                                                300
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gccgcatatg ctcagcatct gaacgatttt gtgacgtata tgaaaaataa tggcgtcaat
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                                                                                                               840
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                                                                                                               960
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                                                                                                              1020
                                                                                                              1080
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                                                                                                              1140
                                                                                                              1200
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<211> 401
<212> PRT
<213> Unknown
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<220>
<223> Obtained from an environmental sample
Page 178

<221> SIGNAL <222> (1)...(32) <400> 246 Met Ser Met Ile Lys Lys Pro Ile Cys Thr Leu Leu Ile Cys Phe Thr Met Leu Ser Val Met Phe Ile Gly Pro Gly Val Thr Glu Val Ser Ala 20 25 30 Ala Asp Ala Asn Ile Asn Ile Asn Ala Glu Arg Gln Val Ile Arg Gly Phe Gly Gly Met Asn His Pro Ala Trp Ile Gly Asp Leu Thr Ala Pro 50 55 60 Gln Arg Glu Thr Ala Phe Gly Asn Gly Gln Asn Gln Leu Gly Phe Ser 65 70 75 80 Ile Leu Arg Ile Phe Val Asp Glu Asn Arg Asn Asn Trp His Arg Glu 85 90 95 Val Ala Thr Ala Lys Arg Ala Ile Glu His Gly Ala Leu Val Ile Ala 100 105 110 Ser Pro Trp Asn Pro Pro Ser Asn Met Val Glu Thr Phe Asn Arg Asn 115 120 125 Gly Thr Ser Ala Lys Arg Leu Arg Tyr Asn Gln Tyr Ala Ala Tyr Ala Gln His Leu Asn Asp Phe Val Thr Tyr Met Lys Asn Asn Gly Val Asn 145 150 160 Leu Tyr Ala Ile Ser Val Gln Asn Glu Pro Asp Tyr Ala His Glu Trp 165 170 Thr Trp Trp Thr Pro Gln Glu Ile Leu Arg Phe Met Arg Glu Asn Ala 180 185 190 Gly Ser Ile Asn Ala Arg Val Ile Ala Pro Glu Ser Phe Gln Tyr Leu 195 200 205 Lys Asn Ile Ser Asp Pro Ile Leu Asn Asp Pro Gln Ala Leu Gly Asn 210 220 Met Asp Ile Leu Gly Ala His Leu Tyr Gly Thr Gln Ile Ser Gln Leu 225 230 240 Pro Tyr Pro Leu Phe Lys Gln Lys Gly Gly Gly Lys Glu Leu Trp Met 245 250 255 Thr Glu Val Tyr Tyr Pro Asn Ser Asp Asn Ser Ala Asp Arg Trp 260 265 270 Pro Glu Ala Leu Gly Val Ser Glu His Ile His His Ser Met Val Glu 275 280 285 Gly Asp Phe Gln Ala Tyr Val Trp Trp Tyr Ile Arg Arg Ser Tyr Gly
290 _____ 295 ___ 300 Pro Met Lys Glu Asp Gly Leu Ile Ser Lys Arg Gly Tyr Asn Met Ala 305 310 315 His Phe Ser Lys Phe Val Arg Pro Gly Tyr Ile Arg Ile Asp Ala Thr 325 330 335 Lys Ser Pro Glu Pro Asn Val Phe Val Ser Ala Tyr Lys Gly Asn Asn 340 345 350 Gln Val Val Ile Val Ala Ile Asn Lys Asn Asn Thr Gly Val Asn Gln 355 360 365 His Phe Val Met Gln Asn Gly Thr Ala Ser Gln Ala Ser Arg Trp Ile 370 Thr Ser Ser Asn Ser Asn Leu Gln Pro Gly Thr Asp Leu Asn Ile Ser 385 390 395 Gly <210> 247 <211> 1044 <212> DNA <213> Unknown <223> Obtained from an environmental sample <400> 247

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                                                                                                                              240
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ctcaagcgca tgcgcgatca catccacacc gtggccggac gctacaaggg caaggtgcgc
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ggctgggacg tggtcaacga ggccttgtcc gacggcggtc ccgaaatcct gcgggattct
                                                                                                                              420
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                                                                                                                              540
                                                                                                                              600
                                                                                                                              660
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                                                                                                                              720
                                                                                                                              780
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ccgctgttgt tcgaccgcgc cctcaagccg aagcccgcgt tcgaggcggt catcaaaaa
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<210> 248 <211> 347

<212> PRT

<213> Unknown

<220>

<223> Obtained from an environmental sample

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1 10 15 Val Ala Gly Glu Ser Glu Ala Gly Lys Leu Ala Gly Ala Gln Phe Ser Ser Val Thr Ala Glu Asn Glu Met Lys Trp Gln Ser Leu His Pro Gln 35 40 45 Pro Asp Arg Tyr Gln Phe Gly Ala Ala Asp Ser Tyr Ile Asp Phe Ala 50 _____ 60 ___ Lys Lys His Lys Met Ala Val Ile Gly His Thr Leu Val Trp His Ser 65 70 75 80 Gln Thr Pro Gly Trp Val Phe Glu Gly Lys Asp Gly Lys Pro Ala Thr 85 90 95 Arg Glu Asp Leu Leu Lys Arg Met Arg Asp His Ile His Thr Val Ala Leu Ser Asp Gly Gly Pro Glu Ile Leu Arg Asp Ser Pro Trp Arg Arg Ile Ile Gly Asp Asp Phe Ile Asp His Ala Phe Arg Phe Ala Arg Glu 145 150 156 150 Ala Asp Pro Lys Ala Glu Leu Tyr Tyr Asn Asp Tyr Gly Leu Glu Asn
165 170 175 Glu Arg Lys Arg Ser Asn Cys Ile Lys Leu Val Lys Gly Met Lys Gln
180 185 190 Arg Gly Val Pro Ile Asp Gly Val Gly Thr Gln Ser His Phe His Leu 200 205 Lys His Pro Ser Leu Gln Glu Ile Glu Lys Thr Ile Lys Asp Phe Ser 210 220 Glu Leu Gly Leu Lys Val Met Ile Thr Glu Leu Asp Val Asp Val Leu 225 230 240 Pro Ser Arg Gly Asn Phe Gly Asn Ala Asp Ile Ser Arg Arg Glu Gln 255 255 Gly Gly Asp Ala Leu Asn Pro Tyr Thr Gly Gly Leu Pro Asp Glu Val 260 265 270 Gln Gln Glu Leu Ala Lys Arg Tyr Ala Asp Ile Phe Asp Ile Tyr Leu 275 280 285 Arg His Arg Lys Ala Val Thr Arg Val Thr Phe Trp Gly Leu Asp Asp 290 295 300 Gly His Thr Trp Leu Asn Gly Phe Pro Ile Arg Gly Arg Thr Asn Tyr 310 315 320 Leu Phe Asp Arg Ala Leu Lys Pro Lys Pro Ala Phe Glu Ala 325 330 335 Val Ile Lys Lys Gly Leu Glu Pro Arg Lys Arg Page 180

340 345

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<213> Unknown
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                                                                                                                                                                           60
                                                                                                                                                                         120
                                                                                                                                                                         180
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                                                                                                                                                                         360
                                                                                                                                                                         420
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Asp Gly Gly Val Glu Lys Asp Leu Phe Gln Ile Leu Lys Asp His Glu
45

Ile Asn Trp Ile Arg Leu Arg Val Trp Asn Asp Pro Arg Asp Glu Asn
50

Gly Asn Pro Leu Gly Gly Gly Asn Cys Asp Tyr Leu Lys Met Thr Glu
65

70

75

80

80
 The Ala Lys Arg Ala Lys Lys Tyr Gly Met Lys Val Leu Leu Asp Phe 85 90 95

His Tyr Ser Asp Trp Trp Ala Asp Pro Gly Lys Gln Tyr Lys Pro Lys 100 105 110
 Glu Trp Asp His Leu His Gly Glu Leu Leu Glu Arg Ala Val Tyr Ser
115 120 125
 Tyr Thr Lys Leu Val Leu Asn His Met Arg Arg Asn Gly Ala Leu Pro
130
140
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Asp Met Val Gln Val Gly Asn Glu Val Asn Asn Gly Phe Leu Trp Pro 145 150 155 160 Asp Gly Met Ile Ala Gly Lys Asp Ala Gly Gly Phe Asp Gly Phe Thr

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Lys Leu Leu Lys Ala Ala Ile Lys Ala Val Arg Glu Val Asp Pro Asp
180 185 190
Ile Lys Ile Val Ile His Leu Ala Glu Gly Gly Asn Asn Ser Leu Phe
Arg Trp Phe Phe Asp Glu Ile Thr Arg Arg Asp Val Asp Phe Asp Val 210 220
Ile Gly Val Ser Tyr Tyr Pro Tyr Trp His Gly Thr Leu Asp Asp Leu
225 230 235 240
                         230
225
Lys Asn Asn Leu Tyr Asp Ile Ala Lys Arg Tyr Asn Lys Asp Val Leu 245 255
The Val Glu Thr Ala Tyr Ala Trp Thr Leu Glu Asp Gly Asp Gly Tyr 260 265 270
Pro Asn Ile Phe Ser Gly Glu Glu Met Glu Leu Thr Gly Gly Tyr Lys 285
Ala Thr Val Gln Gly Gln Ala Thr Phe Leu Arg Asp Leu Ile Glu Val
Val Asn Ser Val Pro Asp Gly His Gly Leu Gly Ile Phe Tyr Trp Glu
305 310 315 320
Gly Asp Trp Ile Pro val Lys Gly Ala Gly Trp Lys Thr Gly Glu Gly
Asn Pro Trp Glu Asn Gln Ala Met Phe Asp Phe Asn Gly Asn Ala Leu 340 345 350
Pro Ser Leu Asp Val Phe Lys Leu Val Arg Thr Val Thr Pro Met Glu 355 ____ 360 365
Ile Lys Ile Glu Glu Ile Leu Pro Val Glu Ile Ser Thr Asn Leu Gly
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Glu Île Pro Lys Phe Pro Asp Ala Val Lys Val Leu Phe Ser Asp Asp 390 395
                         390
Ser Ile Arg Ser Leu Lys Val Thr Trp Asn Phe Asp Pro Ser Leu Val
405 410 415
Glu Thr Pro Gly Val Tyr Arg Val Glu Gly Tyr Val Glu Ser Ile Asp
420
425
430
Gln Lys Ile Phe Ala Thr Leu Thr Val Lys Gly Ser Arg Asn Tyr Leu
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445
Lys Asn Pro Gly Phe Glu Thr Gly Glu Phe Ser Pro Trp Lys Val Phe
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<211> 555
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 <213> Unknown
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                                                                                         300
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                                                                                         480
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Arg Thr Ile Asn Tyr Asn Ala Gly Ile Trp Glu Pro Ser Gly Asn Gly
                                 55
Tyr Leu Thr Leu Tyr Gly Trp Thr Arg Asn Ser Leu Ile Glu Tyr Tyr 65 75 80
Val Val Asp Ser Trp Gly Thr Tyr Arg Pro Thr Gly Thr His Lys Gly
85 90 _ 95
Thr Val Asn Ser Asp Gly Gly Thr Tyr Asp Ile Tyr Thr Thr Met Arg 100 105 110
Tyr Asn Ala Pro Ser Ile Asp Gly Thr Gln Thr Phe Gln Gln Phe Trp
115 120 125
Ser Val Arg Gln Ser Lys Arg Pro Thr Gly Ser Asn Val Ser Ile Thr
130 140
                                                            140
Phe Ser Asn His Val Asn Ala Trp Arg Ser Lys Gly Met Asn Leu Gly 150 155 160
Ser Ser Trp Ser Tyr Gln Val Leu Ala Thr Glu Gly Tyr Gln Ser Ser
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Gly Arg Ser Asn Val Thr Val Trp
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<213> Unknown
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                                                                                                120
                                                                                                180
                                                                                                240
                                                                                                300
ctgtatggct ggaccaccaa tccattggtc gagtactaca tcgtcgacag ctggggtacc
                                                                                                360
taccgtccgc cgggcggcca gggtttcatg ggcacggtag ttagcgacgg gggcacgtac gacgtgtacc ggacgcaacg cgtgaaccag ccatccatca tcggcaacgc cacgttctac
                                                                                                420
                                                                                                480
čagťačtgga gčgtgcggca gtcgaagcgc gtgggcggca ccatcaccat cgccaaccat
                                                                                                540
ttcaacgcct gggccacgct gggcatgaac ctgggccagc acaactacca ggtcatggcc
                                                                                                600
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                                                                                                660
                                                                                                720
780
cagacogiteg egagetegaa ecteaceace ageatgeaga actaeacege etegaceage
                                                                                                840
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gactacatca tcgtgaacgg ccagacccgc cagtccgaag cgcagagcta caacaccggg
ttgtatgcga atggacgctg cggcggtggc tcgaacagcg agtggatgca ttgcaacggc
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                                                                                               960
                                                                                              1020
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                                                                                              1047
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<211> 347
<212> PRT
<213> Unknown
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                                                 10
Leu Gly Ile Thr Ala Ala His Ala Gln Thr Cys Ile Thr Ser Ser Gln
20 25 30
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Thr Gly Thr Asn Asn Gly Asn Tyr Phe Ser Phe Trp Lys Asp Ser Pro \frac{35}{40}
 Gly Thr Val Asn Phe Cys Met Tyr Ala Asn Gly Arg Tyr Thr Ser Asn 50 55 60
 Trp Ser Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Ala Thr Gly 65 70 75 80
 Ser Ser His Thr Ile Ser Tyr Ser Gly Thr Phe Asn Ser Pro Gly Asn 90 95
 Gly Tyr Leu Ala Leu Tyr Gly Trp Thr Thr Asn Pro Leu Val Glu Tyr
100 105 110
 Tyr Ile Val Asp Ser Trp Gly Thr Tyr Arg Pro Pro Gly Gly Gln Gly
115 120 125
 Phe Met Gly Thr Val Val Ser Asp Gly Gly Thr Tyr Asp Val Tyr Arg
 Thr Gln Arg Val Asn Gln Pro Ser Ile Ile Gly Asn Ala Thr Phe Tyr
145 150 160
 Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Val Gly Gly Thr Ile Thr
165 170 175
 Ile Ala Asn His Phe Asn Ala Trp Ala Thr Leu Gly Met Asn Leu Gly
180 185 190
 Gln His Asn Tyr Gln Val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly
195
200
205
 Ser Ser Asp Ile Thr Val Thr Glu Gly Gly Gly Ser Ser Ser Ser Ser Ser 210 220
 Gly Gly Gly Ser Thr Ser Ser Ser Gly Gly Gly Asn Lys Ser Phe
225 230 235 240
Thr Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Asn Ile Gln Leu Gln 245 250 255
Val Asn Asn Gln Thr Val Ala Ser Trp Asn Leu Thr Thr Ser Met Gln 260 265 270
Asn Tyr Thr Ala Ser Thr Ser Leu Ser Gly Gly Ile Thr Val Leu Tyr 275 280 285
Thr Asn Asp Gly Gly Ser Arg Asp Val Gln Val Asp Tyr Ile Ile Val
290 295 300
Asn Gly Gln Thr Arg Gln Ser Glu Ala Gln Ser Tyr Asn Thr Gly 315
Tyr Ala Asn Gly Arg Cys Gly Gly Gly Ser Asn Ser Glu Trp Met His 325 330 335
Cys Asn Gly Ala Ile Gly Tyr Gly Asn Thr Pro
340 345
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<212> DNA
<213> Unknown
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                                                                                        120
cttgccggcc tctacatggc gccggcgaat gcgcaaacct gcatcacgtc gagccagacg
                                                                                        180
ggcaccaaca acggcaacta cttttcgttc tggaaagaca gcccgggcac ggtgaacttc
                                                                                        240
tgcatgtact ccggcggcg ctacacgtcc aactggagcg gcatcaacaa ctgggtgggc
ggcaagggct ggcagacggg ctcgtcccgc accgtctct actccggcag cttcaattcg
ccgggtaacg gctacctgac gctctacggc tggaccacca atccgctcat cgagtactac
atcgtcgaca actggggcag ctatcgtccg ccgggtggcc agggcttcat gggcacggtg
                                                                                        300
                                                                                        360
420
                                                                                        480
aacaccgacg gcggcacgta cgacatctat cgcacgcaac gggtcaacca gccgtcgatc
atcggcaccg cgacgttcta ccagtactgg agcgtgcggc agtcgaagcg caccggcggc
accatcacca cggccaacca cttcaatgcc tgggccagcc tcggcatgaa cctgggacag
                                                                                        540
                                                                                        600
                                                                                        660
cacaactacc aggtgatggc caccgagggc taccagagca gcggcagctc cgacatcacg
                                                                                        720
780
                                                                                        840
                                                                                        900
                                                                                        960
                                                                                       1020
                                                                                       1080
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                                                                                       1137
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<211> 378
<212> PRT
<213> Unknown
<220>
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<222> (1)...(51)
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His Lys Gly Asp Ser Met Ile Phe Gly Leu Lys Ser Ile Thr Gly Arg
Arg Ala Val Ala Ala Leu Ala Cys Leu Ala Gly Leu Tyr Met Ala Pro
Ala Asn Ala Gln Thr Cys Ile Thr Ser Ser Gln Thr Gly Thr Asn Asn 50 60
Gly Asn Tyr Phe Ser Phe Trp Lys Asp Ser Pro Gly Thr Val Asn Phe 65 70 75 80
Cys Met Tyr Ser Gly Gly Arg Tyr Thr Ser Asn Trp Ser Gly Ile Asn 85 90 95
Asn Trp Val Gly Gly Lys Gly Trp Gln Thr Gly Ser Ser Arg Thr Val
Ser Tyr Ser Gly Ser Phe Asn Ser Pro Gly Asn Gly Tyr Leu Thr Leu 115 120 125
Tyr Gly Trp Thr Thr Asn Pro Leu Ile Glu Tyr Tyr Ile Val Asp Asn 130 140
Trp Gly Ser Tyr Arg Pro Pro Gly Gly Gln Gly Phe Met Gly Thr Val
145 150 160
Asn Thr Asp Gly Gly Thr Tyr Asp Ile Tyr Arg Thr Gln Arg Val Asn 165
Gln Pro Ser Ile Ile Gly Thr Ala Thr Phe Tyr Gln Tyr Trp Ser Val
Arg Gln Ser Lys Arg Thr Gly Gly Thr Ile Thr Thr Ala Asn His Phe 200 205
Asn Ala Trp Ala Ser Leu Gly Met Asn Leu Gly Gln His Asn Tyr Gln 210 220 220
Val Met Ala Thr Glu Gly Tyr Gln Ser Ser Gly Ser Ser Asp Ile Thr 225 230 240
Val Trp Glu Gly Thr Ser Ser Gly Gly Ser Ser Asn Gly Gly Ser Ser
245 250 255
                  245
Asn Gly Gly Ser Ser Asn Gly Gly Ser Gly Gly Thr Lys Ser Phe Thr 260 265 270
Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Ser Ile Thr Leu Arg Val
Asn Asn Gln Asn Val Gln Thr Trp Thr Leu Gly Thr Ser Met Gln Asn
290 295 300
Tyr Thr Ala Ser Thr Ser Leu Ser Gly Gly Ile Thr Val Ala Phe Thr 305 310 315 320
Asn Asp Gly Gly Ser Arg Asp Val Gln Val Asp Tyr Ile Ile Val Asn 325 330 335
Gly Gln Thr Arg Gln Ser Glu Gln Gln Ser Tyr Asn Thr Gly Leu Tyr 340 345 350
Ala Asn Gly Ser Cys Gly Gly Gly Ser Asn Ser Glu Trp Met His Cys
355 360 365
 Asn Gly Ala Ile Gly Tyr Gly Asn Thr Pro
370
 <210> 257
 <211> 2694
 <212> DNA
 <213> Unknown
 <223> Obtained from an environmental sample
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<400> 257

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gcctatttcc tggaccagta tggcaagaag acgatttcca gcgtcatggc caatgtcaac tggaacaaca cttgtgccga gaaagtctat aaactcacgg gcaagtatcc tgccatgaac tgctacgact tcatccacat ctgtttctcg ccagccaact ggattgacta caccgacatc
 actcctgcca aggaatggca cgatgcgggc ggtatcgtac agttgatgtg gcatttcaat gtgcctaaga gccagggggc aacagatgtt acctgcacgc ccagcgagac cacctttaag
agctacgaca gccagaagaa cctcaccggc taccattgga taaaggcca ggagttccgc aactacggcc agctgctgct gttcgagggc tataacgaga tgctcgatgc caacaactcc tggaattttg cacagagcag ttcagcctac gatgccatca acaaatacgc ccagagcttt gtcgatgtcg tacgcgcac cggtggcaac aatgcccagc gcaacctcat tgtcagcaca tacggcgcct gctcaggcaa cggcacgtgg gatgcaagag tgcaagaccc cttgaagaaa ctgcaggattc ccacgggtga aagcaaccat atcatcttcg aggttcacaa ctatccctcc atcgtcaaca aggacaacgc gggcaactac gtcagcgatc gcaacacacac gcaagagattg atgcatgggt taagaactac gtcagcgatc gcacacacag gggcaactac gtcagcgatc gcaacacaag gcagagattg gcaagaggt taagaactaa aagacccacc gcaatcacag gcgaatgggg caccaacaac gtcgatgccg gcggtggcaa gacagactac gacctccata aggacctgat gtcgaattt gtcagctaca tgataaagac catgaagcag aacgacattg ccaccttcta ctggatggga atgctgcag gcgctccacg gcgctccacg gcgcttcacac agccctcaca agcccgacct ggcgctgaag atgctgcag gcgctccacg gcgctccacg cacctaccc gccttcacac agcccgacct ggcgctgaag atgctgcag cctatcacgg cgactcttgg aatccctacc tgcctgacgc caaggacttt cccgaaggca aaatcacctc ggccacggtg aattccaaca gccaatgggg cgaactgacc atccacgatg gagctattga caagaccgtc tatagaggta tcaaggtgga gctggaagaa aagcctgcca ctggagccct gtcttcaag gtatatgca acagtgagaa ggcaacagcc atcaattcca aaaccccaca gttggctttc ttcagtaca agagcgtcaa ccttatacag cacgacgact ccacgaacc ctgtagtctg
  atcaaaatca agagcgtcaa ccttatcaag cacgacgact ccacagaacc ctgtagtctg
  aaagtggctt ggggttgtac tctcagcgac cagaactacg ccacgggcat cgaagacatt actatcactc ctgttcgtca tgacgatgga atcatctaca atctgagcgg acagcctgta
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  <210> 258
<211> 897
  <212> PRT
  <213> Unknown
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<400> 258
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Ser Ser Val Met Ala Asn Val Asn Trp Asn Asn Thr Cys Ala Glu Lys 35
Val Tyr Lys Leu Thr Gly Lys Tyr Pro Ala Met Asn Cys Tyr Asp Phe 50
Ile His Ile Cys Phe Ser Pro Ala Asn Trp Ile Asp Tyr Thr Asp Ile 65
Thr Pro Ala Lys Glu Trp His Asp Ala Gly Gly Ile Val Gln Leu Met 90
Page 186
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Trp His Phe Asn Val Pro Lys Ser Gln Gly Ala Thr Asp Val Thr Cys 105 Thr Pro Ser Glu Thr Thr Phe Lys Ala Ser Asn Ala Leu Val Ser Gly
115 120 125 Thr Trp Glu Asn Lys Trp Phe Tyr Glu Gln Met Asp Lys Val Ile Ala 130 135 140 Thr Ile Leu Lys Leu Gln Asp Ala Gly Ile Ala Ala Thr Trp Arg Pro
145 150 160 Phe His Glu Ala Ala Gly Asn Ala Cys Ala Lys Gln Gln Ala Asp Trp
165 170 175 Thr Lys Ala Trp Phe Trp Trp Gly Tyr Asp Gly Ala Asp Thr Tyr Lys Lys Leu Trp Ile Ala Met Tyr Asp Tyr Phe Lys Leu Lys Gly Val Asn 195 200 _ 205 Asn Leu Ile Trp Met Trp Thr Thr Gln Asn Tyr Asn Gly Asp Ser Ser 210 220 Lys Tyr Asn Gln Asp Thr Asp Trp Tyr Pro Gly Asp Glu Tyr Val Asp 225 230 235 240 The Val Ala Arg Asp Leu Tyr Gly Tyr Asn Ala Asp Gln Asn Leu Gln 245 250 255 Glu Phe Ser Glu Ile Gln Ala Ala Tyr Pro Asn Lys Met Val Val Leu 260 265 270 Gly Glu Cys Gly Lys Gly Asp Ser Gly Asp Pro Gly Lys Met Ser Asp 275 280 285 Val Trp Ala Lys Gly Ala Lys Trp Gly His Phe Met Val Trp Tyr Gln Gly Glu Gln Gly Ser Thr Asp Thr Met Cys Ser Asp Asp Trp Trp Lys 315 320 Asp Ala Met Ser Ser Ala Asn Val Ile Thr Arg Asp Lys Val Val Ile 325 330 335 Pro Asp Val Thr Ser Thr Ile Glu Asn Ala Thr Asp Ala Val Lys Asn 340 345 350 Met Gly Leu Gly Trp Asn Leu Gly Asn Ala Leu Asp Ala Asn Ala Gln 355 ____ 360 365 Gln Tyr His Asp Ala Thr Gln Asp Asn Tyr Trp Gly Gln Gln Asp Ile 370 380 Thr Ser Glu Ser Cys Trp Gly Gln Leu Pro Thr Lys Ala Glu Leu Met 385 390 395 400 Ala Met Met Lys Glu Ala Gly Phe Gly Ala Ile Arg Val Pro Val Thr 405 410 415 Trp Tyr Asn His Met Asp Lys Asp Gly Asn Val Asp Ala Ala Trp Met Asn Arg Val His Glu Val Val Asp Tyr Val Ile Ser Gln Gly Met Tyr
435 440 445 Cys Ile Leu Asn Val His His Asp Thr Gly Ala Asp Ser Tyr Asp Ser 450 460 Gln Lys Asn Leu Thr Gly Tyr His Trp Ile Lys Ala Asp Glu Thr Asn 465 470 475 480 Tyr Ala Thr Asn Lys Ala Arg Tyr Glu Lys Leu Trp Gln Gln Ile Ala 485 490 495 Gln Glu Phe Arg Asn Tyr Gly Gln Leu Leu Leu Phe Glu Gly Tyr Asn 500 505 Glu Met Leu Asp Ala Asn Asn Ser Trp Asn Phe Ala Gln Ser Ser Ser 515 520 525 Ala Tyr Asp Ala Ile Asn Lys Tyr Ala Gln Ser Phe Val Asp Val Val Arg Ala Thr Gly Gly Asn Asn Ala Gln Arg Asn Leu Ile Val Ser Thr 545 550 560 Tyr Gly Ala Cys Ser Gly Asn Gly Thr Trp Asp Ala Arg Val Gln Asp 565 570 575 Pro Leu Lys Lys Leu Gln Ile Pro Thr Gly Glu Ser Asn His Ile Ile 580 585 590 Phe Glu Val His Asn Tyr Pro Ser Ile Val Asn Lys Asp Asn Ala Gly 600 605 Asn Tyr val Ser Asp Arg Thr Ĭle Ser Glu Ile Lys Ala Glu Ile Asp 610 620 Ala Trp Leu Lys Asn Leu Lys Thr His Leu Val Ser Lys Gly Ala Pro 625 630 640 Val Ile Ile Gly Glu Trp Gly Thr Asn Asn Val Asp Ala Gly Gly Page 187

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Lys Thr Asp Tyr Asp Leu His Lys Asp Leu Met Phe Glu Phe Val Ser
660 665 670
Tyr Met Ile Lys Thr Met Lys Gln Asn Asp Ile Ala Thr Phe Tyr Trp
675 680 685
                                680
         675
Met Gly Leu Thr Asp Gly Ala Pro Arg Thr Tyr Pro Ala Phe Thr Gln 690 700
Pro Asp Leu Ala Leu Lys Met Leu Gln Ala Tyr His Gly Asp Ser Trp
705 710 715 720
Asn Pro Tyr Leu Pro Asp Ala Lys Asp Phe Pro Glu Gly Lys Ile Thr
725 730 735
Ser Ala Thr Val Asn Phe Asn Ser Gln Trp Gly Glu Leu Thr Ile His 740 745 750
Asp Gly Ala Ile Asp Lys Thr Val Tyr Arg Gly Ile Lys Val Glu Leu
755 760 765
Glu Glu Lys Pro Ala Thr Gly Ala Leu Ser Phe Lys Val Tyr Ala Asn
770 775 780
Ser Glu Lys Ala Thr Ala Ile Asn Ser Lys Thr Pro Gln Leu Ala Phe
785 790 795 800
Phe Ser Tyr Thr Gly Ile Gln Lys Ile Asn Leu Gln Trp Asn Ile Ala
805 810 815
Thr Lys Gly Ser Ile Lys Ile Lys Ser Val Asn Leu Ile Lys His Asp 820 825 830
Asp Ser Thr Glu Pro Cys Ser Leu Lys Val Ala Trp Gly Cys Thr Leu 835
Ser Asp Gln Asn Tyr Ala Thr Gly Ile Glu Asp Ile Thr Ile Thr Pro
Val Arg His Asp Asp Gly Ile Ile Tyr Asn Leu Ser Gly Gln Pro Val 865 870 880
Thr Ser Pro Gln Arg Gly Ile Tyr Ile Leu Asn Gly Lys Lys Ile Ile
885 890 895
<210> 259
<211> 1143
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<223> Obtained from an environmental sample.

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<210> 260 <211> 380 <212> PRT <213> Unknown

60 120

960

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<220>
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<221> SIGNAL
<222> (1)...(24)
<400> 260
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Met Phe Leu Leu Ser Cys Gln Ala Gln Lys Pro Val Asp Ser Leu Lys 20 25 30
Glu Ala Phe Asp Gly Leu Phe Leu Ile Gly Thr Ala Met Asn Thr Pro 35 40 45
Gln Ile Thr Gly Gln Asp Thr Gln Thr Leu Glu Leu Ile Lys Lys His
50 55 60
Met Asn Ser Ile Val Ala Glu Asn Val Met Lys Ser Glu Val Leu Gln
65 70 75 80
Pro Arg Glu Gly Glu Phe Asp Phe Thr Leu Ala Asp Gln Phe Val Gln 85 90 95
Phe Gly Ile Asp Asn Asn Met His Ile Val Gly His Thr Leu Ile Trp
100 105 110
His Ser Gln Ala Pro Arg Trp Phe Phe Val Asp Glu Asn Gly Asn Asp
115 120 125
Val Ser Pro Glu Ile Leu Lys Gln Arg Met Lys Asp His Ile Tyr Thr
val val Gly Arg Tyr Lys Gly Lys Ile His Gly Trp Asp Val Val Asn
145 150 155 160
145
Glu Cys Ile Asn Asp Asp Gly Ser Trp Arg Asn Ser Lys Phe Tyr Gln
165 170 175
                    165
Ile Leu Gly Glu Asp Phe Val Lys Tyr Ala Phe Gln Phe Ala Ala Glu
180 185 190
Ala Asp Pro Asp Ala Glu Leu Tyr Tyr Asn Asp Tyr Ser Met Phe Leu
195 200 205
Pro Gly Arg Arg Glu Gly Val Ile Lys Met Val Arg Asn Leu Gln Glu 210 220
Gln Gly Ile Lys Ile Asp Gly Ile Gly Met Gln Gly His Leu Met Ile
225 230 235 240
Asp Tyr Pro Pro Leu Glu Asp Phe Glu Thr Ser Ile Leu Ala Phe Ala
                                            250
Asp Leu Gly Val Asn Val Met Ile Thr Glu Leu Asp Ile Ser Val Leu 260 _ _ _ 265 _ _ 270 _ _ _
Pro Phe Pro Thr Arg Asn Val Gly Ala Asp Val Ser Leu Asn Ile Ala
275 280 285
Tyr Asn Thr Glu Leu Asn Pro Tyr Pro Asn Gly Leu Pro Glu Asp Val
290 295 300
     290
                              295
Ala Gln Lys Leu His Asn Arg Trp Val Asp Leu Phe Arg Leu Phe Ile
305 310 315 320
Lys His His Asp Lys Ile Thr Arg Val Thr Thr Trp Gly Thr Ala Asp 325 330 335
Ala Met Ser Trp Lys Asn Asn Trp Pro Ile Arg Gly Arg Thr Asp Tyr 340 345
Pro Leu Leu Phe Asp Arg Asp Phe Gln Pro Lys Pro Phe Val Ala Asp 355 360 365
Ile Ile Lys Glu Ala Leu Ala Ala Lys Arg Lys Leu
370 375 380
<210> 261
<211> 1629
<212> DNA
 <213> Unknown
<223> Obtained from an environmental sample.
<400> 261
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                                                                                       120
                                                                                       180
                                                                                       240
                                              Page 189
```

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cccggtggta gaagggttgt tcactatcaa ggccaatata atgttgataa ttcacaaaac
                                                                                                              300
 tcttatttgg cattgtatgg ctggacacgc tcaccactga ttgaatatta cgtgattgaa
                                                                                                              360
 agttacggct cgtataaccc gtcgaattgc acccaaggtc ggcagaccta tggcaccttt
                                                                                                              420
 cagagtgatg gtgcaaccta tgaaattgtt cgctgtcagc gagttcagca gccctctatc gatggcacac aaactttcta tcaatacttc agtgtggctc agccgaagaa aggctttggt agtatcagtg gtacgatcac tgtgggcaac catttgatg catgggccgc cgccggtttg
                                                                                                              480
                                                                                                              540
                                                                                                              600
 aacctggggg aacatgatta tatggtgatg gctaccgagg gttatcagag caccggtagt
                                                                                                              660
 tcggatatta cggtcagtga aattaccggt ggttcaggtg gtggctcttc ctcgggtgct aataccctgg tgattcgtgc tgtgggcacc tctggtaatg aattgctgcg tgtcaatgtg ggtggtagcc ctgtgcagac attgagcctt tcgaccagtt ggcaggattt tactgtcaat acggatgcaa cgggtgacat taacgtagag ttgtttaatg atcagggtca gggttatgag
                                                                                                              720
                                                                                                             780
                                                                                                             840
                                                                                                             900
 gcgcgtatcg attatgtgct ggttaatggt gagacccgct acgcggccga tcagagttat
                                                                                                             960
 gatggcatga cctgggacgg cgaatgtggg ggtggctctt ttacccagtg gatgcattgt gatggcatga ttggctttgg tgatatgacc ggcggcaatg ccggtggtgg cggttcttcg ggtggttctg gcgccaatac tctggtggtg cgtgctgtcg gcacttcagg taacgagcag
                                                                                                            1020
                                                                                                            1080
                                                                                                            1140
 ttgcgcgtga atgtgggcgg caacacgatt caaacactga acctgtcaag cagttggcaa gattttactg tcaataccga tgcctcgggc gatattaacg tagagctgtt taatgaccag
                                                                                                            1200
                                                                                                            1260
 ggtcagggct atgaggcgcg tattgattat gtgctggtta atggcgagac ccgctacgcg gctgaccaga gttataacac cagcgcctgg gatggcgaat gcgggggtgg ctcttttacc caatggatgc attgtgatgg catgattggt tttggtgata tgtcgggtgg tggttctgct gtgggtacaa gcagtagcgg taatgccggc agcaatacca gcagtgcctg ttactgtaat tggggataggca gtgtgatggc ttcttgtgaa aatcagtga acggctgggg ttggggaaaat
                                                                                                            1320
                                                                                                            1380
                                                                                                            1440
                                                                                                            1500
                                                                                                            1560
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                                                                                                            1620
 tgcaattaa
                                                                                                            1629
 <210> 262
<211> 542
 <212> PRT
 <213> Unknown
· <220>
 <223> Obtained from an environmental sample.
 <221> SIGNAL <222> (1)...(26)
 <400> 262
Met Ile Asn Lys Ile Gly Lys Gly Phe Phe Ser Ala Phe Ile Cys Ala \frac{1}{1} \frac{1}{1} \frac{1}{1} \frac{1}{1}
Ala Ala Leu Ser Val Ser Thr Val Asn Ala Gln Gln Thr Val Thr Thr 20 25 30
Asn Thr Gln Gly Thr His Asp Gly Phe Phe Tyr Ser Phe Trp Lys Asp
Ser Gly Asp Ala Ser Phe Gly Leu Arg Glu Gly Gly Arg Tyr Thr Ser
Gln Trp Asn Thr Ser Thr Asn Asn Trp Val Gly Gly Lys Gly Trp Asn 65 75 80
Pro Gly Gly Arg Arg Val Val His Tyr Gln Gly Gln Tyr Asn Val Asp 85 90 95
Asn Ser Gln Asn Ser Tyr Leu Ala Leu Tyr Gly Trp Thr Arg Ser Pro
Leu Ile Glu Tyr Tyr Val Ile Glu Ser Tyr Gly Ser Tyr Asn Pro Ser
115 120 125
Asn Cys Thr Gln Gly Arg Gln Thr Tyr Gly Thr Phe Gln Ser Asp Gly
130
140
Ala Thr Tyr Glu Ile Val Arg Cys Gln Arg Val Gln Gln Pro Ser Ile
145 150 155 160
Asp Gly Thr Gln Thr Phe Tyr Gln Tyr Phe Ser Val Arg Gln Pro Lys
165 170 175
Lys Gly Phe Gly Ser Ile Ser Gly Thr Ile Thr Val Gly Asn His Phe
180
185
190
Asp Ala Trp Ala Ala Ala Gly Leu Asn Leu Gly Glu His Asp Tyr Met
Val Met Ala Thr Glu Gly Tyr Gln Ser Thr Gly Ser Ser Asp Ile Thr
```

Val ser Glu Ile Thr Gly Gly Ser Gly Gly Ser Ser Ser Gly Ala 225 230 Asn Thr Leu Val Ile Arg Ala Val Gly Thr Ser Gly Asn Glu Leu Leu 245 250 Page 190

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Arg Val Asn Val Gly Gly Ser Pro Val Gln Thr Leu Ser Leu Ser Thr
                   260
                                               265
 Ser Trp Gln Asp Phe Thr Val Asn Thr Asp Ala Thr Gly Asp Ile Asn 275 280 285
 Val Glu Leu Phe Asn Asp Gln Gly Gln Gly Tyr Glu Ala Arg Ile Asp 290 295 300
      Val Leu Val Asn Gly Glu Thr Arg Tyr Ala Ala Asp Gln Ser
 Asn Thr Ser Ala Trp Asp Gly Glu Cys Gly Gly Gly Ser Phe Thr Gln
325 335
 Trp Met His Cys Asp Gly Met Ile Gly Phe Gly Asp Met Thr Gly Gly 340 345 350
 Asn Ala Gly Gly Gly Ser Ser Gly Gly Ser Gly Ala Asn Thr Leu 355
 Val Val Arg Ala Val Gly Thr Ser Gly Asn Glu Gln Leu Arg Val Asn 370 380
 Val Gly Gly Asn Thr Ile Gln Thr Leu Asn Leu Ser Ser Ser Trp Gln 385 _____ 400
 Asp Phe Thr Val Asn Thr Asp Ala Ser Gly Asp Ile Asn Val Glu Leu 405
 Phe Asn Asp Gln Gly Gln Gly Tyr Glu Ala Arg Ile Asp Tyr Val Leu
420 430
 Val Asn Gly Glu Thr Arg Tyr Ala Ala Asp Gln Ser Tyr Asn Thr Ser
 Ala Trp Asp Gly Glu Cys Gly Gly Gly Ser Phe Thr Gln Trp Met His
 Cys Asp Gly Met Ile Gly Phe Gly Asp Met Ser Gly Gly Gly Ser Ala
475 480
 Val Gly Thr Ser Ser Gly Asn Ala Gly Ser Asn Thr Ser Ser Ala
485 490 495
 Cys Tyr Cys Asn Trp Tyr Gly Ser Val Met Ala Ser Cys Glu Asn Gln 500 510
 Val Asn Gly Trp Gly Trp Glu Asn Asn Gln Ser Cys Ile Gly Asn Asn 515
 Thr Cys Asn Asn Gln Gly Gly Ser Gly Gly val Cys Asn
530 540
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 <211> 1092
 <212> DNA
 <213> Unknown
 <220>
 <223> Obtained from an environmental sample.
<400> 263
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gtgcttggca tacaggcttc aatcgcacag gaaatttgta ttaccagcgg cactgaccag atcagagaaa ccacatccaa cggctatacc cacgaactat ggaatcagga cacccggggg acggcctgta tgactattaa tgcaggcacc acttacagtg cgcggtggaa cggtgcattt aactatttgg cccgccgtgg attggcctac gatggtcgt ccctcaccca tgctgaccgg gggaaattca ccataaatta tgcctctaac tacaactgca acaatatgaa tgggctctt
                                                                                                      60
                                                                                                     120
                                                                                                     180
                                                                                                    240
                                                                                                     300
                                                                                                    360
tatttaagcg tgtacggatg gacgcgggat tttgccaagg aaaatgccaa tccggcagga tcacaggctc atcaggaagc gctggtggaa tattacattg ttgaaaactg gtgcgactgg aatgtttcac aagaccctaa cgcccagagt ctgggcaccc tgaatgttga tgggtcgatc
                                                                                                    420
                                                                                                    480
                                                                                                    540
tatgatatgt atcgcacaga acggatcaac caaccttcta tcaggtgcgg tggtacctgc
                                                                                                    600
gataatttīt accaatacīt cagcattcgc cgcaacacac gtaācagtgg cāccattgāt
                                                                                                    660
gtcagcgctc atttcaacca gtgggaagca ttaaccggcg tccctatggg tggcctgcac gaagtgatga tgaaggtcga aggctacaac tcaaacaatc aatccagtgg caatgtaagc
                                                                                                    720
                                                                                                    780
tttactcaat tgctcatgcg tgcccgcttc gaggatggcg ccattgtcga gaaccagaat
                                                                                                    840
gcggtcggcc atgcgcacgg tggagaagcg gtgggagatg atcaccgccg tcttgccctg
ggccaggccc ttgaagcggg cgaacacctc ggcctcggcc ttggcgtcga gggcggcggt
gggttcgtcg agaatgatca actcggcgtc gcgcatatag gcgcgggcga tggctacctt
ctgccactcg ccgcccgaga ggtcgcggcc ctgcttgaaa aggcgccca attgctccag
                                                                                                    900
                                                                                                    960
                                                                                                   1020
                                                                                                   1080
agaaacgggt ga
                                                                                                   1092
<210> 264
<211> 363
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<212> PRT

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<213> Unknown
 <220>
 <223> Obtained from an environmental sample.
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Leu Ala Leu Leu Val Leu Gly Ile Gln Ala Ser Ile Ala Gln Glu Ile
20 25 30
Cys Ile Thr Ser Gly Thr Asp Gln Ile Arg Glu Thr Thr Ser Asn Gly
Tyr Thr His Glu Leu Trp Asn Gln Asp Thr Arg Gly Thr Ala Cys Met
50
60
Thr Ile Asn Ala Gly Thr Thr Tyr Ser Ala Arg Trp Asn Gly Ala Phe 65 70 _____ 75 ___ 80
Asn Tyr Leu Ala Arg Arg Gly Leu Ala Tyr Asp Gly Ser Ser Leu Thr
85 90 95
His Ala Asp Arg Gly Lys Phe Thr Ile Asn Tyr Ala Ser Asn Tyr Asn 100 105 110
Cys Asn Asn Met Asn Gly Leu Ser Tyr Leu Ser Val Tyr Gly Trp Thr
Arg Asp Phe Ala Lys Glu Asn Ala Asn Pro Ala Gly Ser Gln Ala His
130 135 140
Gln Glu Ala Leu Val Glu Tyr Tyr Ile Val Glu Asn Trp Cys Asp Trp
145 150 155 160
Asn Val Ser Gln Asp Pro Asn Ala Gln Ser Leu Gly Thr Leu Asn Val
Asp Gly Ser Ile Tyr Asp Met Tyr Arg Thr Glu Arg Ile Asn Gln Pro
180 _ _ _ 185 _ _ 190
Ser Ile Arg Cys Gly Gly Thr Cys Asp Asn Phe Tyr Gln Tyr Phe Ser
Ile Arg Arg Asn Thr Arg Asn Ser Gly Thr Ile Asp Val Ser Ala His
Phe Asn Gln Trp Glu Ala Leu Thr Gly Val Pro Met Gly Gly Leu His 235 235 240
Glu Val Met Met Lys Val Glu Gly Tyr Asn Ser Asn Asn Gln Ser Ser
245 250 255
Gly Asn Val Ser Phe Thr Gln Leu Leu Met Arg Ala Arg Phe Glu Asp 260 270
Gly Ala Ile Val Glu Asn Gln Asn Ala Val Gly His Ala His Gly Gly 275 280 285
Glu Ala Val Gly Asp Asp His Arg Arg Leu Ala Leu Gly Gln Ala Leu
290 295 300
Glu Ala Gly Glu His Leu Gly Leu Gly Leu Gly Val Glu Gly Gly 305 310 320
Gly Phe Val Glu Asn Asp Gln Leu Gly Val Ala His Ile Gly Ala Gly 325 330 335
Asp Gly Tyr Leu Leu Pro Leu Ala Ala Arg Glu Val Ala Ala Leu Leu 340 350
Glu Lys Ala Pro Gln Leu Leu Gln Arg Asn Gly
<210> 265
<211> 996
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample.
<400> 265
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                                                                                  60
gcggtcaatc ctgtgacgat cgagatgcaa aaacagttgt tgatcgatca tgtcaacagt
                                                                                 120
attacggcag agaaccatat gaagtttgag catcttcagc cggaagaagg gaaatttacc
tttcaggaag cggatcggat tgtggatttt gcttgttcgc accgaatggc ggttcgaggg
                                                                                 180
                                                                                 240
                                          Page 192
```

```
cacacacttg tatggcacaa ccagactccg gattgggtgt ttcaagatgg tcaaggccat ttcgtcagtc gggatgtgtt gcttgagcgg atgaaatgtc acattcaac tgttgtacgg cgatacaagg gaaaaatata ttgttgggat gtcatcaacg aagcggtagc cgacgaagga gacgaattgt tgaggccgtc gaagtggcga caaatcatcg gggacgatt tatggaacaa gcattctct acgcttatga agctgaccca gatgcactgc tttttacaa tgactataat
                                                                                                                                                     300
                                                                                                                                                     360
                                                                                                                                                     420
                                                                                                                                                     480
                                                                                                                                                     540
gaatgttttc cggaaaagāg agaaāaaatt īttgcactīg tcaaatcgct gcgtgataaa
                                                                                                                                                     600
ggcattccga ticatggcat cggcatgcag gcgcactgga gcctgacccg cccgtcgctt gatgaaattc gtgcggcgat tgaacggtat gcgtccttg gtgttgttct tcatattacg gaactcgatg tatccatgtt tgaatttcac gatcgtcgaa ccgattggc tgtcccgacg aacgaaatga tcgaacagca agcagaaacgg tatgggcaaa ttittgcttt gtttaaggag
                                                                                                                                                    660
                                                                                                                                                    720
                                                                                                                                                    780
                                                                                                                                                    840
tatogogatg ttattcaaag tgtcacattt tggggaattg ctgatgacca tacatggctc
                                                                                                                                                    900
gataacttic cagigcacgg gagaaaaac iggccgciit igiicgaiga acagcataaa
                                                                                                                                                    960
ccgaaaccag ctttttggcg ggcagtgagt gtctga
                                                                                                                                                    996
<210> 266
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<211> 331 <212> PRT

<213> Unknown

<220s

<223> Obtained from an environmental sample.

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100 105 110 Cys His Ile Ser Thr Val Val Arg Arg Tyr Lys Gly Lys Ile Tyr Cys
115 120 125 Trp Asp Val Ile Asn Glu Ala Val Ala Asp Glu Gly Asp Glu Leu Leu 130 140 Arg Pro Ser Lys Trp Arg Gln Ile Ile Gly Asp Asp Phe Met Glu Gln 145 155 160 Ala Phe Leu Tyr Ala Tyr Glu Ala Asp Pro Asp Ala Leu Leu Phe Tyr 165 170 Asn Asp Tyr Asn Glu Cys Phe Pro Glu Lys Arg Glu Lys Ile Phe Ala 180 185 190 Leu Val Lys Ser Leu Arg Asp Lys Gly Ile Pro Ile His Gly Ile Gly 195 200 205 Met Gln Ala His Trp Ser Leu Thr Arg Pro Ser Leu Asp Glu Ile Arg 210 220 Ala Ala Ile Glu Arg Tyr Ala Ser Leu Gly Val Val Leu His Ile Thr 225 230 235 240 Glu Leu Asp Val Ser Met Phe Glu Phe His Asp Arg Arg Thr Asp Leu 245 250 255 Ala Val Pro Thr Asn Glu Met Ile Glu Gln Gln Ala Glu 260 265 Gln Ile Phe Ala Leu Phe Lys Glu Tyr Arg Asp Val Ile Gln Ser Val 280 Thr Phe Trp Gly Ile Ala Asp Asp His Thr Trp Leu Asp Asn Phe Pro 290 295 300 Val His Gly Arg Lys Asn Trp Pro Leu Leu Phe Asp Glu Gln His Lys 315 320 Pro Lys Pro Ala Phe Trp Arg Ala Val Ser Val

<210> 267 <211> 1956 <212> DNA

<213> Bacteria

<400> 267
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acacatggag gctacgacta tgagctctgg aaagactacg gaaatacgat tatggaactt
aacgacggtg gtacttttag ttgtcaatgg agtaatatcg gtaatgcact atttagaaaa
gggagaaaat ttaattccga caaaacctat caagaattag gagatatagt gacaagaatac
ccactggttg aatattacat tgtagaaagc tggggcagct tattatgagac
ccacaaggaa ccatcacaat ggatggcggt acttatgaaac tatatgaaac tggagacaca
accacagcctt ccatcgatgg aactgcgaca ttccaacaat attggagtgt tcgtacatcc
aaggaaacaa gcggaacaat atctgtcact gaacattta aacagtggga aagaatgggc
atgcgaatgg gtaagatgta tgaagttgct cttaccgttg aaggttatca
ccaaagcccaa tatataagaa tgaaatcaga ataggtgcaa atccaactcc
caaagcccaa ttagaagaga tgcattttca ataatcgaag cggaagaata taacagcaca
aatcctcca ctttacaagt gattggaacg ccaaataatg gcagaggaat tggttatatt <400> 267 gttgactggt ttgtattctc aaaatcagga acttaa

<210> 268

<211> 651 <212> PRT

<213> Bacteria

<220>

<221> SIGNAL <222> (1)...(30)

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Gly Arg Lys Phe Asn Ser Asp Lys Thr Tyr Gln Glu Leu Gly Asp Ile 90

Val Val Glu Tyr Gly Cys Asp Tyr Asn Pro Asn Gly Asn Ser Tyr Leu 100

Cys Val Tyr Gly Trp Thr Arg Asn Pro Leu Val Glu Tyr Tyr Ile Val 115

Glu Ser Trp Gly Ser Trp Arg Pro Pro Gly Ala Thr Pro Lys Gly Thr 130

Ile Thr Val Asp Gly Gly Thr Tyr Glu Ile Tyr Glu Thr Thr Arg Val 145

Asn Gln Pro Ser Ile Asp Gly Thr Ala Thr Phe Gln Gln Tyr Trp Ser 165

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Val Arg Thr Ser Lys Arg Thr Ser Gly Thr Ile Ser Val Thr Glu His
180 185 190 Phe Lys Gln Trp Glu Arg Met Gly Met Arg Met Gly Lys Met Tyr Glu 195 200 205 Val Ala Leu Thr Val Glu Gly Tyr Gln Ser Ser Gly Tyr Ala Asn Val 210 220 Tyr Lys Asn Glu Ile Arg Ile Gly Ala Asn Pro Thr Pro Ala Pro Ser 225 230 240 Gln Ser Pro Ile Arg Arg Asp Ala Phe Ser Ile Ile Glu Ala Glu Glu 245 250 255 Tyr Asn Ser Thr Asn Ser Ser Thr Leu Gln Val Ile Gly Thr Pro Asn 260 265 270 Asn Gly Arg Gly Ile Gly Tyr Ile Glu Asn Gly Asn Thr Val Thr Tyr 275 280 285 Ser Asn Ile Asp Phe Gly Ser Gly Ala Thr Gly Phe Ser Ala Thr Val Ala Thr Glu Val Asn Thr Ser Ile Gln Ile Arg Ser Asp Ser Pro Ile 305 310 315 320 Gly Thr Leu Ceu Gly Thr Leu Tyr Val Ser Ser Thr Gly Ser Trp Asn 325 330 335 Thr Tyr Gln Thr Val Ser Thr Asn Ile Ser Lys Ile Thr Gly Val His 340 350 Asp Ile Val Leu Val Phe Ser Gly Pro Val Asn Val Asp Asn Phe Ile 355 ____360 ____365 Phe Ser Arg Ser Ser Pro Val Pro Ala Pro Gly Asp Asn Thr Arg Asp 370 375 Ala Tyr Ser Ile Ile Gln Ala Glu Asp Tyr Asp Ser Ser Tyr Gly Pro Asn Leu Gln Ile Phe Ser Leu Pro Gly Gly Gly Ser Ala Ile Gly Tyr 405 410 415 Ile Glu Asn Gly Tyr Ser Thr Thr Tyr Asn Asn Val Asn Phe Ala Asn 420 425 430 Gly Leu Ser Ser Ile Thr Ala Arg Val Ala Thr Gln Ile Ser Thr Ser 435 440 445 Ile Gln Val Arg Ala Gly Gly Ala Thr Gly Thr Leu Leu Gly Thr Ile
450
455
460 Tyr Val Pro Ser Thr Asn Ser Trp Asp Ser Tyr Gln Asn Val Thr Ala
465 470 475 480 Asn Leu Ser Asn Ile Thr Gly Val His Asp Ile Thr Leu Val Phe Ser 485 490 495 Gly Pro Val Asn Val Asp Tyr Phe Val Phe Thr Pro Ala Asn Val Asn 500 510 Ser Gly Pro Thr Ser Pro Val Gly Gly Thr Arg Ser Ala Phe Ser Asn 515 520 525 Ile Gln Ala Glu Asp Tyr Asp Ser Ser Tyr Gly Pro Asn Leu Gln Ile
530
540 Phe Ser Leu Pro Gly Gly Gly Ser Ala Ile Gly Tyr Ile Glu Asn Gly 545 550 560 Tyr Ser Thr Thr Tyr Lys Asn Ile Asp Phe Gly Asp Gly Ala Thr Ser 565 570 575 Val Thr Ala Arg Val Ala Thr Gln Asn Ala Thr Thr Ile Gln Val Arg
580 585 590 Leu Gly Ser Pro Ser Gly Thr Leu Leu Gly Thr Ile Tyr Val Gly Ser Thr Gly Ser Phe Asp Thr Tyr Arg Asp Val Ser Ala Thr Ile Ser Asn 610 620 Thr Ala Gly Val Lys Asp Ile Val Leu Val Phe Ser Gly Pro Val Asn 625 630 635 640 635 Val Asp Trp Phe Val Phe Ser Lys Ser Gly Thr 645 650

<210> 269 <211> 1110

<212> DNA

<213> Unknown

<223> Obtained from an environmental sample.

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                                                                                                  120
 cagtgcgcga cctggacccg aagcaccatt cgcaattgcg agggcatcga ctacgagttg tggaaccaga acaaccgcgg cacggtcaac atggaaatca cgggaaacgg aacgttcgcg
                                                                                                  180
                                                                                                  240
 gcgacgtgga gcggaacgga aaacatcctg tttcgcgccg gcaagaaatg ggggttcaac
                                                                                                  300
 agcaccacga cggcgcggtc ggtcggcgcc atcacgctcg atttcgctgc gacctggacc
                                                                                                  360
 tccagcgaca acgtgaaaat gctcggcatc tacggctggg cgtattaccc gtcgggaagc gagccgacga aaacggaaag cggtcaaaac acgagctttt ccgatcagat cgagtattac atcatccagg accgcgggagg cttcaacccg ggttccggcg gcgtcaacgc caaaaagtac ggcgaggccg tgatcgacgg aatcgcctat gacttttggg tggccgaccg gatcaaccag
                                                                                                  420
                                                                                                  480
                                                                                                  540
                                                                                                  600
cccatgctga caggaaggg caacttcaag caatacttca gcgttccacg gaacacgagc agccaccggc aaagcggcat cgtcagcatt tcgaagcact ttgaggagtg ggacaaggcc ggcatgaaga tgctggactg tccgctatac gaagtcgcga tgaaggtgga atcgtatacg ggctcggcga atggcgggggt ggcccggaag gtcggcgga cccggcggt ccgccgaaag catgcggggt ccgccgaaag catgcggggt
                                                                                                  660
                                                                                                  720
                                                                                                  780
                                                                                                  840
                                                                                                  900
 gccttcgttc aggaaagagt gctcaaggtc gcgcccgtcg acggaacccg cctgcaagtc
                                                                                                  960
 aaggtgcggg acgtgaaggg cgtgaaccgg gccgagttca atgccgcggg cgcggcaacg
ttctcgttgt cccatgtccc cgcgggcccg tatttcctgg atgtgacggg gccggatgta
                                                                                                1020
                                                                                                1080
 agacagatca cgccgttcgt tttgcgataa
                                                                                                1110
 <210> 270
 <211> 369
 <212> PRT
 <213> Unknown
<223> Obtained from an environmental sample.
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1 10 15
Val Leu Gly Val His Lys Val Phe Tyr Ala Ala Leu Ala Cys Val Ala
20 25 30
Met Gly Tyr Ser Glu Thr Trp Ala Gln Cys Ala Thr Trp Thr Arg Ser
Thr Ile Arg Asn Cys Glu Gly Ile Asp Tyr Glu Leu Trp Asn Gln Asn 50 55 60
Asn Arg Gly Thr Val Asn Met Glu Ile Thr Gly Asn Gly Thr Phe Ala 70 75
Ala Thr Trp Ser Gly Thr Glu Asn Ile Leu Phe Arg Ala Gly Lys Lys
Trp Gly Phe Asn Ser Thr Thr Thr Ala Arg Ser Val Gly Ala Ile Thr 100 _ 105 _ 110
Leu Asp Phe Ala Ala Thr Trp Thr Ser Ser Asp Asn Val Lys Met Leu 115 120 125
Gly Ile Tyr Gly Trp Ala Tyr Tyr Pro Ser Gly Ser Glu Pro Thr Lys
130
135
140
Thr Glu Ser Gly Gln Asn Thr Ser Phe Ser Asp Gln Ile Glu Tyr Tyr 150 155 160
Ile Ile Gln Asp Arg Gly Gly Phe Asn Pro Gly Ser Gly Gly Val Asn 165 170 175
Ala Lys Lys Tyr Gly Glu Ala Val Ile Asp Gly Ile Ala Tyr Asp Phe
180
185
190
Trp Val Ala Asp Arg Ile Asn Gln Pro Met Leu Thr Gly Arg Gly Asn 195 200 205
Phe Lys Gln Tyr Phe Ser Val Pro Arg Asn Thr Ser Ser His Arg Gln 210 220
Ser Gly Ile Val Ser Ile Ser Lys His Phe Glu Glu Trp Asp Lys Ala
225 230 235 240
Gly Met Lys Met Leu Asp Cys Pro Leu Tyr Glu Val Ala Met Lys Val 245 250 255
Glu Ser Tyr Thr Gly Ser Ala Asn Gly Gly Gly Ser Ala Asn Val Thr
Arg Asn Ile Leu Thr Leu Gly Gly Ser Ser Ala Pro Thr Pro Ile Ala 275
Arg Gly Pro Gly Arg Ser Ala Glu Ser Met Arg Val Ala Phe Val Gln 290 300
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Glu Arg Val Leu Lys Val Ala Pro Val Asp Gly Thr Arg Leu Gln Val

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305
                                310
 Lys Val Arg Asp Val Lys Gly Val Asn Arg Ala Glu Phe Asn Ala Ala 325 330 335
 Gly Ala Ala Thr Phe Ser Leu Ser His Val Pro Ala Gly Pro Tyr Phe 340 345 350
 Leu Asp Val Thr Gly Pro Asp Val Arg Gln Ile Thr Pro Phe Val Leu
                                             360
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 <210> 271
<211> 1128
 <212> DNA
 <213> Unknown
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 <400> 271
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 atgggagaaa acatgacact acaagaagcc tttgccgatc acttttatgt gggagccgcc
                                                                                                              120
 atcagccaac gccttttca accagatcgc gccgaaacgc tgcaactggc cgcgcaccaa ttcaacagca tcacagccga aaatgagatg aagtggcagt cgttaaatcc cactcctggc
                                                                                                              180
                                                                                                              240
 gaataccott tcgaaaacoc cgataaattc gtccocttig gigtcgaaaa cgatatgiac
gatactiff togalactic cyaladatic gittyctity gigityadad tyalatytac atceptings acgitetett ctggcacage cagacacceg actggetett caaggatgac gacggtaact tegteteeg egaagtetta etegacegea tgegegeea egtgegeaat ettgteeage getaeggeaa ceatgtgeae geetgggatg ttateaatga aaceetteaat gataatggtt cettgegega caageeetga acgeaaatee tegagegagga atteategag caeggeettee ggattgeegg egaggaacte eeeeetgataatgat
                                                                                                              300
                                                                                                              360
                                                                                                              420
                                                                                                              480
                                                                                                              540
                                                                                                              600
tattcgatga ccattcctgc caagcgcgat gctgttgctg aaatggttcg cgacctcata gccaaaggca tccgcattga cggcgttggc atgcagggac attgggcacg gacccacccg accatagcgg acatagaaaa aagcattct gccttcgcag gaaccggcgt acaggtacac atcactgagc tcgacatcga catgctgcca cgccatcccc agatggttcac tggtggtgca
                                                                                                              660
                                                                                                              720
                                                                                                              780
                                                                                                              840
gacaccatgt tgcgcctaca acaagatccc aaactcgacc cctacactga gggacttcca gcggaagatc agcaggcatt ggcagaacgc tacgcaagca tcttccgttt attcttgaag cacagcgatg ttattcgccg tgtcaccttc tggggggtca ccgatgccca cacctggctc aacaattggc ccatccgtgg ccgcaccagc catcccctgc tcttcgaccg ccagaacaac
                                                                                                              900
                                                                                                              960
                                                                                                            1020
                                                                                                            1080
 cccaaacccg ccttccacgc cgtcgtcaga ctgaagaccg aagactga
                                                                                                            1128
 <210> 272
 <211> 375
 <212> PRT
 <213> Unknown
<223> Obtained from an environmental sample.
<221> SIGNAL
<222> (1)...(22)
 <400> 272
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Ala Thr Ala Thr Met Gly Glu Asn Met Thr Leu Gln Glu Ala Phe Ala 20 25 30
Asp His Phe Tyr Val Gly Ala Ala Ile Ser Gln Arg Leu Phe Gln Pro
                                           40
Asp Arg Ala Glu Thr Leu Gln Leu Ala Ala His Gln Phe Asn Ser Ile 50 60
Thr Ala Glu Asn Glu Met Lys Trp Gln Ser Leu Asn Pro Thr Pro Gly 65 70 75 80
Glu Tyr Arg Phe Glu Asn Ala Asp Lys Phe Val Arg Phe Gly Val Glu
                                                        90
Asn Asp Met Tyr Ile Val Gly His Val Leu Phe Trp His Ser Gln Thr
                                                 105
Pro Asp Trp Leu Phe Lys Asp Asp Gly Asn Phe Val Ser Arg Glu 115
Val Leu Leu Asp Arg Met Arg Ala His Val Arg Asn Leu Val Gln Arg
130 140
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Tyr Gly Asn His Val His Ala Trp Asp Val Ile Asn Glu Thr Phe Asn
                              150
                                                             155
Asp Asn Gly Ser Leu Arg Asp Ser Pro Trp Thr Gln Ile Leu Gly Glu
165 170 175
Glu Phe Ile Glu His Ala Phe Arg Ile Ala Gly Glu Glu Leu Pro Pro
180 185 190
His Val Glu Leu Leu Tyr Asn Asp Tyr Ser Met Thr Ile Pro Ala Lys
                                           200
                                                                         205
Arg Asp Ala Val Ala Glu Met Val Arg Asp Leu Ile Ala Lys Gly Ile 210 220
Arg Ile Asp Gly Val Gly Met Gln Gly His Trp Ala Arg Thr His Pro
Thr Ile Ala Asp Ile Glu Lys Ser Ile Leu Ala Phe Ala Gly Thr Gly 245 250 255
Val Gln Val His Ile Thr Glu Leu Asp Ile Asp Met Leu Pro Arg His 260 270
Pro Gln Met Phe Thr Gly Gly Ala Asp Thr Met Leu Arg Leu Gln Gln 275 280 285
Asp Pro Lys Leu Asp Pro Tyr Thr Glu Gly Leu Pro Ala Glu Asp Gln
290 295 300
Gln Ala Leu Ala Glu Arg Tyr Ala Ser Ile Phe Arg Leu Phe Leu Lys
305 310 315 320
His Ser Asp Val Ile Arg Arg Val Thr Phe Trp Gly Val Thr Asp Ala
His Thr Trp Leu Asn Asn Trp Pro Ile Arg Gly Arg Thr 340
                                                                               350
Leu Leu Phe Asp Arg Gln Asn Asn Pro Lys Pro Ala Phe His Ala Val 355 360 365
Val Arg Leu Lys Thr Glu Asp
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<211> 1134
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample.
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                                                                                                          120
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                                                                                                          240
                                                                                                          300
                                                                                                          360
                                                                                                          420
                                                                                                          480
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                                                                                                         780
                                                                                                         840
                                                                                                         900
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                                                                                                         960
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<211> 377
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<213> Unknown
<220>
<223> Obtained from an environmental sample.
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<221> SIGNAL

<222> (1)...(74)

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100 105 110 Asp Gln Ile Thr Pro Glu Asn Glu Gly Lys Trp Gly Ser Val Glu Gly
115 120 125 Thr Arg Asp Gln Tyr Asn Trp Ala Pro Leu Asp Arg Ile Tyr Asp Tyr
130
135
140 Ala Arg Gln His Asn Ile Pro Val Lys Ala His Thr Leu Val Trp Gly
145 150 155 160 Ala Gln Ala Pro Gly Trp Ile Asn Asn Leu Ser Ala Ala Glu Gln Arg
165 170 175 Glu Glu Ile Glu Glu Trp Ile Arg Asp Tyr Cys Thr Arg Tyr Pro Asp
180 185 190 Thr Gln Met Ile Asp Val Val Asn Glu Ala His Pro Asn His Ala Pro
195 200 205 Ala Arg Tyr Ala Gln Asn Ala Phe Gly Asn Asp Trp Ile Thr Glu Ala 210 220 Phe Lys Leu Ala Arg Arg His Cys Pro Asn Ala Ile Leu Ile Tyr Asn 225 235 240 Asp Tyr Asn Phe Ile Thr Trp Asp Thr Asp Glu Ile Met Ala Leu Ile 245 250 255 Arg Pro Ala Ile Ala Ala Gly Val Val Asp Ala Val Gly Leu Gln Ala 260 265 270 His Ser Leu Tyr Pro Asp Glu Tyr Ala Asn Lys Met Trp Ser Ala Ala Glu Ile Gln Gln Lys Leu Asp Leu Ile Ser Thr Leu Gly Val Pro Met 290 295 _ 300 Tyr Ile Ser Glu Tyr Asp Val Ala Lys Ser Asn Asp Gln Glu Gln Leu 305 310 315 320 Ala Ile Phe Ser Glu Gln Phe Pro Val Leu Tyr Glu His Pro Asn Val 325 330 Val Gly Val Thr Leu Trp Gly Tyr Ile Asp Gly Ala Thr Trp Arg Ala 340 345 350 Gly Ser Gly Leu Ile Arg Asn Gly Gln His Arg Pro Ala Met Gln Trp 355 360 365 Leu Leu Glu Tyr Leu Glu Asn Asn Arg 370 375

<210> 275 <211> 1401 <212> DNA

<213> Unknown

<220>

<223> Obtained from an environmental sample.

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Page 199

540

600

900

960

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ggtgttccag gcggttctaa tccaacgcag ggacaatggg cgaatcctaa aacagcaaga
 gttctaaaac taggacctga tatgacaagt gtggtaggca gcgcatcaac cattgatgct
 Ccttttatgt ttgaagattc ggggatgcat aagtataacg gaacctatta ctattcctat tgcatcaact ttggcggctc ccacccagca gataaaccac ctggtgagat cggttatatg acgagtcaa gtccgatggg tccctttacg tatagagggc acttcctgaa aaatccgggt gcattttcg ggggaggcgg taataaccac catgctgtt tcaccttaa aaaccgagtgg
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                                                                                                             1020
                                                                                                              1080
                                                                                                              1140
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                                                                                                              1200
                                                                                                              1260
                                                                                                             1320
                                                                                                              1380
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                                                                                                             1401
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 <211> 466
 <212> PRT
 <213> Unknown
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 <223> Obtained from an environmental sample.
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15
Tyr Met Ser Ser Asp Asp Tyr Glu Tyr His Ser Asn Gly Thr Ile Lys 20 25 30
Asp Asn Ser Phe Ala Asn Leu Asn Arg Val Phe Val Ile Ser Ser Ala 35 40 45
Asp Met Val Asn Trp Thr Asp His Gly Ala Ile Pro Val Ala Gly Ala 50 60 60
Asn Gly Ala Asn Gly Gly Lys Gly Ile Ala Lys Trp Ala Gly Ala Ser
Trp Ala Pro Ser Ala Ala Val Lys Lys Ile Ash Gly Lys Asp Lys Phe 85 90 95
Phe Leu Tyr Phe Ala Asn Ser Gly Gly Gly Ile Gly Val Leu Thr Ala
100
105
110
Asp Ser Pro Ile Gly Pro Trp Thr Asp Pro Ile Gly Lys Ala Leu Val
115
Thr Pro Asp Thr Pro Gly Mot Ala Cly Val Val Val Val Try
Thr Pro Asn Thr Pro Gly Met Ala Gly Val Val Trp Leu Phe Asp Pro
Ala Val Phe Val Asp Asp Asp Gly Thr Gly Tyr Leu Tyr Ala Gly Gly
145 150 155
Gly Val Pro Gly Gly Ser Asn Pro Thr Gln Gly Gln Trp Ala Asn Pro
165 170 175
Lys Thr Ala Arg Val Leu Lys Leu Gly Pro Asp Met Thr Ser Val Val 180 185 190
Gly Ser Ala Ser Thr Ile Asp Ala Pro Phe Met Phe Glu Asp Ser Gly
195 200 205
Met His Lys Tyr Asn Gly Thr Tyr Tyr Tyr Ser Tyr Cys Ile Asn Phe 210 220
Gly Gly Ser His Pro Ala Asp Lys Pro Pro Gly Glu Ile Gly Tyr Met 225 230 235 240
Thr Ser Ser Pro Met Gly Pro Phe Thr Tyr Arg Gly His Phe Leu 245 250 255
Lys Asn Pro Gly Ala Phe Phe Gly Gly Gly Gly Asn Asn His His Ala 260 265
Val Phe Asn Phe Lys Asn Glu Trp Tyr Val Val Tyr His Thr Gln Thr
275
280
285
Val Ser Ser Ala Leu Tyr Gly Ser Gly Lys Gly Tyr Arg Ser Pro His 290 295 300
Ile Asn Lys Leu Val His Asn Ala Asp Gly Ser Leu Arg Glu Val Ala 305
Ala Asn Phe Glu Gly Val Lys Gln Leu Ser Asn Leu Asn Pro Tyr Gln
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Arg Val Glu Ala Glu Thr Phe Ala Trp Asn Gly Arg Ile Leu Thr Glu

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Ala Ser Ser Ala Pro Gly Gly Pro Val Asn Asn Gln His Val Thr Asn
            355
                                        360
                                                                     365
Ile Gln Asn Gly Asp Trp Val Ala Ala Ser Asn Val Asp Phe Gly Ser
                                  375
      370
                                                               380
Asn Gly Ala Arg Thr Phe Lys Ala Asn Val Ala Ser Asn Thr Gly Gly 385 390 395 400
Lys Ile Glu Val Arg Leu Gly Ser Pro Asp Gly Arg Leu Val Gly Thr
                       405
                                                    410
Leu Asn Val Pro Ser Thr Gly Gly Thr Asn Asn Trp Arg Glu Val Glu
420 425 430
Thr Ala Val Asn Gly Ala Ala Gly Val His Asn Val Phe Phe Val Phe
           435
                                       440
                                                                    445
Thr Gly Thr Gly Ala Asn Leu Phe Gln Phe Asp Ser Trp Gln Phe Thr
     45Ó
                                  455
                                                               460
Gln Arg
465
<210> 277
<211> 1128
<212> DNA
<213> Unknown
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                                                                                                      60
                                                                                                    120
gcgctgaatt ctgagcagat attgggtcgg gatacacgcg gactcgaatt gattagaact cattttaacg ccattacgcc cgaaacatt accaaatggg aggctatcca tcccgaaccc ggtgtctatg attttaaaga ggctgatgca ttcgtcgatt ttggccaaaa atataatatg ttcatggtgg gtcatacact ggtttggcat agtcagacac cgctgggt cttcaaagac
                                                                                                    180
                                                                                                    240
                                                                                                    300
                                                                                                    360
gaaaatggcg cgttggtatc gcgcgaggta ctgttagagc ggatgcgcga ccacatccac
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accettette eccettacce tegacetatt caceectege atetcetaaa ceaaeccetc
                                                                                                    480
aatgaagacg gttcgtacag agaaacactg tggtaccaaa taattggtac ggactatatt cttaaagcat tcgaatttgc ccgggaggcc gatcccgacg ctgagctata ctataacgat tactcgcttg agaacccctc aaaggagagcc ggcgcgatgc gaattgttca atacctgcag
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                                                                                                    660
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                                                                                                   1080
                                                                                                   1128
<210> 278
<211> 375
<212> PRT
<213> Unknown
<223> Obtained from an environmental sample.
<221> SIGNAL
<222> (1)...(19)
<400> 278
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Cys Ser Ala Gly Asn Gln Asp Arg Val Pro Ser Leu His Ala Glu Phe
Ser Asp Ala Phe Leu Ile Gly Thr Ala Leu Asn Ser Glu Gln Ile Leu
Gly Arg Asp Thr Arg Gly Leu Glu Leu Ile Arg Thr His Phe Asn Ala
Ile Thr Pro Glu Asn Ile Thr Lys Trp Glu Ala Ile His Pro Glu Pro 65 75 80
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Gly Val Tyr Asp Phe Lys Glu Ala Asp Ala Phe Val Asp Phe Gly Gln
Lys Tyr Asn Met Phe Met Val Gly His Thr Leu Val Trp His Ser Gln
                100
                                             105
                                                                        110
Thr Pro Arg Trp Val Phe Lys Asp Glu Asn Gly Ala Leu Val Ser Arg
Glu Val Leu Leu Glu Arg Met Arg Asp His Ile His Thr Val Val Gly
                                 135
Arg Tyr Arg Gly Arg Ile His Gly Trp Asp Val Val Asn Glu Ala Leu
145 _ _ _ 150 _ _ 155 _ 160
Asn Glu Asp Gly Ser Tyr Arg Glu Thr Leu Trp Tyr Gln Ile Ile Gly
165 170 175
                                                  170
Thr Asp Tyr Ile Leu Lys Ala Phe Glu Phe Ala Arg Glu Ala Asp Pro
180 185 190
Asp Ala Glu Leu Tyr Tyr Asn Asp Tyr Ser Leu Glu Asn Pro Ser Lys
200 205
Arg Ala Gly Ala Met Arg Ile Val Gln Tyr Leu Gln Glu His Gly Ala
                                 215
Pro Ile Thr Gly Val Gly Thr Gln Gly His Phe Thr Leu Asp Trp Pro 225 230 240
Glu Leu Ser Glu Ile Glu Gln Thr Val Ile Asp Phe Ala Ser Leu Gly 245 250 255
Met Asp Val Met Ile Thr Glu Leu Asp Ile Asp Val Leu Pro Gln Pro 260 _ _ 270
Asp Asp Tyr Thr Gly Ala Asp Val Asn Phe Ser Ala Glu Leu Tyr Asp 275 280 285
Glu Leu Asn Pro Trp Pro Asn Gly Leu Pro Pro Glu Ile Glu Gln Glu
290 ___ 295 ___ 300
Leu Ala Asn Arg Tyr Ala Asp Ile Phe Glu Ile Tyr Leu Arg His Arg 305 310 315 320
Asp Lys Val Thr Arg Val Ser Phe Trp Gly Val Thr Asp Gly Asp Ser
325 ____330 ___335
Trp Lys Asn Asn Trp Pro Val Pro Gly Arg Thr Asn Tyr Pro Leu Ile 340 350
Phe Asp Arg Asn Trp Lys Pro Lys Pro Ala Phe Phe Ser Ile Val Asp 355 360 365
                                       360
Ala Ala Arg Glu Ala Leu Asp
370 375
<210> 279
<211> 786
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample.
<400> 279
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                                                                                                  60
                                                                                                 120
180
aacttcaatg cgatcaccgc ggagaatgtg atgaaatggg aggccgttca tccccggccg
                                                                                                 240
ggagaatata cgttcggcgc cgcggaccgg ttcgttgagt tcggggaaaa gaacggcctg
ttcatcgtgg ggcacacgct gatctggcat tctcaaacgc cggcctgggt tttcgaggat
gagaatggcg cgccgctcgg ccgcgaggcg ctgctggag ggatgggcga tcacatcac
                                                                                                 300
                                                                                                 360
                                                                                                 420
accyttyccy gacyttacay gygccytyty aaggygtygy acytygtcaa cyaagccctc
                                                                                                 480
gccgaggacg gttccctgcg ggattcgccg tggcgccgca tcataggcga cgactattc
gtgaaggcct ttgagtttgc gcgggaagct gatccggatg cggagttgta ttacaacgat
tactcgattg aaaacgaacc gaagcgcaag ggggcggtgg cgttggtgag gacgctccag
gcggcggtgg ttcccgttgc cggcgtgggg attcaggcaa acggcaatc ccattggct
                                                                                                 540
                                                                                                 600
                                                                                                 660
                                                                                                 720
tctccgcggc ttgtcgaaga ggcgatccgg gactttgcca gtttgggcgt caaggtgatg
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atctga
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<210> 280
<211> 261
<212> PRT
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<213> Unknown

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<221> SIGNAL
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Leu Phe Ala Ala Ser Ala Thr Pro Glu Thr Thr Leu Lys Asp Ala Phe
                                          25
Ala Asp His Phe Leu Val Gly Ala Ala Leu Asn Glu Ser His Phe Ala
                                    40
Glu His Asn Pro Ala His Ala Gly Leu Val Ala Ala Asn Phe Asn Ala
50 55 60
Ile Thr Ala Glu Asn Val Met Lys Trp Glu Ala Val His Pro Arg Pro 65 70 75 80
Gly Glu Tyr Thr Phe Gly Ala Ala Asp Arg Phe Val Glu Phe Gly Glu
Lys Asn Gly Leu Phe Ile Val Gly His Thr Leu Ile Trp His Ser Gln
                                         105
Thr Pro Ala Trp Val Phe Glu Asp Glu Asn Gly Ala Pro Leu Gly Arg
115 120 125
Glu Ala Leu Leu Glu Arg Met Arg Asp His Ile His Thr Val Ala Gly
130 135 140
Arg Tyr Arg Gly Arg Val Lys Gly Trp Asp Val Val Asn Glu Ala Leu
                                                   155
                         150
Ala Glu Asp Gly Ser Leu Arg Asp Ser Pro Trp Arg Arg Ile Ile Gly
165 170 175
                    165
Asp Asp Tyr Phe Val Lys Ala Phe Glu Phe Ala Arg Glu Ala Asp Pro
                                         185
Asp Ala Glu Leu Tyr Tyr Asn Asp Tyr Ser Ile Glu Asn Glu Pro Lys
195 _ _ _ 200 _ 205 _ _ _ _ 205
Arg Lys Gly Ala Val Ala Leu Val Arg Thr Leu Gln Ala Ala Gly Val
210 215 220
Pro Val Ala Gly Val Gly Ile Gln Gly His Gly Asn Leu His Trp 230 235
Ser Pro Arg Leu Val Glu Glu Ala Ile Arg Asp Phe Ala Ser Leu Gly 255
Val Lys Val Met Ile
               260
<210> 281
<211> 963
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample.
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                                                                                           180
                                                                                           240
                                                                                           300
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ccctccctgc aggcggtcga gaacgcgttc aaaaagatct ccgccctggg gctcaagctg
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gtgtggggcg tatgcgacgg caccagctgg atcggcgcga gctatcccct ccccttgac
                                                                                           900
ġccgġġċtgc gtcccaagcc ctccttcttc ggcatactcc ġcgcccttga cgaacagaac
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                                                                                           963
tga
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<210> 282

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<211> 320
<212> PRT
<213> Unknown
<220>
<223> Obtained from an environmental sample.
<400> 282
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Glu Leu Ile Arg Thr Gln Tyr Ser Ile Ile Thr Pro Glu Asn Glu Leu
20 25 30
Lys Pro Asp Ser Val Leu Asp Val Ala Ala Ser Arg Ala Leu Ala Lys
Glu Asp Asp Thr Ala Val Ala Val His Phe Ser Ala Ala Ala Pro Ile 50 _____ 60
Leu Asn Phe Ala Arg Asp Asn Gly Ile Lys Val His Gly His Val Leu 70 75 80
Val Trp His Ser Gln Thr Pro Glu Glu Phe Phe His Glu Gly Tyr Asn 85 _ 90 _ 95
Ala Ser Ala Pro Tyr Val Ser Arg Glu Val Met Leu Ala Arg Leu Asp
100 105 110
Asn Tyr Ile Arg Leu Ile Phe Glu Tyr Met Asp Glu Asn Tyr Pro Gly
115
120
125
Leu Ile Val Ser Trp Asp Val Ala Asn Glu Cys Val Ala Asp Gly Ser
130 140
Thr Ala Leu Arg Thr Ser Asn Trp Thr Arg Val Val Gly Gln Asp Phe 150 155 160
Val Ala Arg Ala Phe Glu Ile Ala Asp Lys Tyr Ala Pro Glu Asp Val
165 170 ____ 175
Met Leu Cys Tyr Asn Asp Tyr Ser Thr Pro Tyr Glu Pro Lys Leu Thr
Gly Ile Val Asn Leu Leu Thr Glu Leu Thr Gln Glu Gly His Ile Asp
195 200 205
Gly Tyr Gly Phe Gln Ser His Tyr Ser Val Gly Asp Pro Ser Leu Gln 210 220
Ala Val Glu Asn Ala Phe Lys Lys Ile Ser Ala Leu Gly Leu Lys Leu
225 _ 230 _ 240
Arg Val Ser Glu Leu Asp Ile Lys Val Asp Ala Asp Ser Glu Pro Asn
245 250 255
Arg Ala Leu Gln Ala Asp Arg Tyr Glu Ala Leu Leu Arg Ile Tyr Met 260 270
Lys Tyr Gly Val Ser Ala Val Gln Val Trp Gly Val Cys Asp Gly Thr
Ser Trp Ile Gly Ala Ser Tyr Pro Leu Pro Phe Asp Ala Gly Leu Arg
Pro Lys Pro Ser Phe Phe Gly Ile Leu Arg Ala Leu Asp Glu Gln Asn 315 310 320
<210> 283
<211> 4161
<212> DNA
<213> Unknown
<223> Obtained from an environmental sample.
<400> 283
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                                                                                       120
gaaaaccaag gaaactatgt ccaatctggt ggtgcgaccc tcactctagt aaaaaacaaa
                                                                                       180
gtgtttgcag ggaatgaaga tggaactgca ctatatatta gtaatcgatc gaataactgg gacggggcag atttccgttt cacggatctt ggattacaag atggaaaaac atatacgatc aatattatag gatatgtcga tgaaaatgaa gttgttcctt caggagccca agtgtattg
                                                                                       240
                                                                                       300
                                                                                      360
420
caaactgtag ataaaacata tggatggtta gcaagcgcgg acttaaaaaa cggagagtcg
ttcactataa atacaacgtt cacccttgac atgagtaaag gggacacccg tcttcgtata
                                                                                       480
caatccaacg atagtggtaa aaaagtttca ttttacgtcg ggtattttc aatttcaatt agtgatgtag aaggagaaga tggtgggagc tctatttcaa ggccaccggc tttacctttt gaaactattg actttgaaga tcaaagttta agtggagagaggaggagc aggcacggaa
                                                                                       540
                                                                                       600
                                                                                      660
                                             Page 204
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cauctttcta	cccsantcon	aantonaant	antacasatt	ataacacaag	tttaagtaaa	840
ntaattanto	ttastasta	adgiggdagi	totagegagee	acaacaacac	citaaytaaa	900
gradicaging	atttassass	atyggtacty	Latyaayyaa	agrategeta	caatagttcg	960
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435 440 445 Thr Thr Arg Glu Asp Gly Ser Pro Leu Gly Arg Glu Glu Ala Leu Glu 450 460 Asn Leu Lys Asn His Ile Glu Thr Val Met Lys His Phe Gly Asp Arg Val Ile Ser Trp Asp Val Val Asn Glu Ala Ile Ile Asp Asn Pro Pro Asn Pro Asp Asn Trp Glu Glu Ser Leu Arg Lys Ser Pro Trp Tyr Tyr 500 505 Ser Ile Gly Ser Asp Tyr Val Glu Gln Ala Phe Arg Ile Ala Arg Gln 515 520 525

Val Leu Asp Glu Asn Gly Trp Asp Ile Lys Leu Tyr Tyr Asn Asp Tyr 530 540 Asn Glu Asp Asn Gln Arg Lys Ala Gln Ala Ile Tyr His Met Val Lys 545 550 555 560 Glu Leu Asn Glu Lys Tyr Ala Gln Glu His Pro Gly Lys Arg Leu Ile Asp Gly Ile Gly Met Gln Gly His Tyr Ser Ile Arg Thr Asn Pro Asp 580 585 590 Asn Val Lys Met Ser Leu Glu Arg Phe Ile Ser Leu Gly Val Glu Val 595 600 605 Ser Ile Thr Glu Leu Asp Ile Gln Ala Gly Thr Asp Asn His Leu Thr 610 620 Glu Glu Gln Ser Lys Ala Gln Ala Tyr Leu Tyr Ala Lys Leu Phe Lys 625 630 635 Ile Phe Lys Glu Asn Ala Ser His Ile Ser Arg Val Thr Leu Trp Gly
645 650 655 Leu Asn Asp Ala Ala Ser Trp Arg Ala Ser Thr Ser Pro Leu Leu Phe 660 670 Asp Arg Asn Leu Gln Ala Lys Pro Ser Tyr Tyr Ala Val Île Asp Pro 675 680 685 Asp Thr Phe Ile Glu Glu Asn Pro Thr Val Thr Glu Glu Ser Arg Lys 690 _ 695 700 Ala Ile Ala Leu Tyr Gly Ile Pro Val Ile Asp Gly Ser Ile Asp Ser 705 710 715 720 Ile Trp Glu Ser Val Pro Tyr Ile Pro Ile Asp Arg Tyr Gln Met Ala 725 730 735 Trp Gln Gly Ala Ser Gly Thr Ala Lys Val Leu Trp Asp Glu Gly Asn 740 750 Leu Tyr Val Leu Val Gln Val Asn Asp Asp Gln Leu Asp Lys Ser Ser 765 760 765 Thr Asn Pro Trp Glu Gln Asp Ser Ile Glu Val Phe Val Asp Glu Asn 770 775 780 Asn Ala Lys Thr Ser Phe Tyr Gln Glu Asp Asp Gly Gln Tyr Arg Val 785 790 795 800 Asn Phe Asp Asn Glu Thr Ser Phe Asn Pro Pro Ser Ile Glu Asn Gly 805 810 Phe Met Ser Glu Thr Asn Val Ser Gly Thr Asn Tyr Val Val Glu Met 820 825 830 Lys Ile Pro Leu Arg Ser Ile Gln Leu Lys Asn Gly Ser Glu Ile Gly 835 840 845 Phe Asp Val Gln Ile Asn Asp Gly Lys Asn Gly Ala Arg Gln Ser Val 850 855 860 Ala Ala Trp Asn Asp Thr Thr Gly Thr Ala Tyr Met Asp Thr Ser Val 865 870 875 880 Phe Gly Thr Leu Thr Leu Leu Thr Thr Leu Asp Asn Glu Asn Thr Pro Gly Ser Gly Thr Thr Pro Gly Ser Gly Thr Thr Pro Gly Ser Gly Thr 900 905 910 Thr Pro Gly Ser Ser Thr Thr Pro Gly Ser Gly Thr Thr Pro Gly Ser 915 Gly Thr Thr Pro Gly Ser Gly Thr Thr Pro Gly Ser Gly Thr Thr Pro 930 940 Gly Ser Gly Thr Thr Pro Gly Ser Gly Thr Thr Pro Gly Ser Gly Thr 950 955 960 Thr Pro Gly Ser Gly Thr Thr Pro Gly Ser Gly Thr Thr Pro Gly Ser 975 Gly Thr Thr Pro Val Lys Gly Glu Asn Gly Thr Val Val Leu Gln Pro 980 985 990 Lys Val Glu Thr Lys Glu Lys Asp Gly Lys Val Val Glu Lys Val Ala 995 1000 1005 Thr Ile Ser Thr Asn Glu Val Glu Ala Ile Val Lys Glu Leu Ser Asn 1010 1025 1020
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1095
1100

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1115
1120
 Glu Phe His Val Val Ala Lys Ala Asn Gly Lys Glu Arg Val Ile Asp
1125 1130 1135
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1235 1240 1245
 Leu Pro Ser Glu Thr Tyr Asp Gly Arg Phe Ala Asp Val Lys Gly Thr
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Glu Trp Phe Asn Lys Asn Gly Glu Leu Ala Ala Ala Val Lys Phe Gly
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Glu Ala Val Tyr Gln Ala Gly Ile Met Gln Gly Arg Asp Ser Gly Asn
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Glu Glu Ala Glu Arg Val Gly Met Thr Val Tyr Gly His Thr Leu Ala
65 70 75 80
Trp His Ser Gln Gln Gln Asn Ala Tyr Leu Asn Gly Leu Ile Lys Gly
85 90 95
Lys Lys Thr Glu Val Glu Pro Gly Gln Glu Ser Glu Val Leu Leu
100 105 110
Gln Thr Asp Phe Asn Asp Gly Asn Val Thr Phe Asn Gly Trp Gly Asn 115 120 125
Asn Ser Ser Arg Thr Val Glu Asn Gly Ala Leu Lys Leu Thr Asn Pro
130 135 140
Ser Val Val Asn Ser Trp Glu Ala Gln Phe Ala Tyr Asp Phe Ser Glu
145 150 160
Ala Phe Glu Met Asp Lys Thr Tyr Lys Leu Lys Phe Arg Ile Lys Gly
165 170 175
Ser Ala Ala Gly Lys Ile Ala Ala Gly Phe Gln Ile Thr Asp Gly Tyr
180 185 190
Leu Ser Ala Gly Glu Phe Gly Thr Val Glu Phe Asn Thr Gln Trp Lys 200 205
Asp Val Glu Leu Ser Cys Val Cys Ser Ala Glu Gly Gly Thr Arg Leu
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Ile Phe Ser Phe Gly Glu Phe Ala Gly Asp Ile Tyr Ile Asp Asp Phe 225 230 240
Cys Phe Ser Val Glu Gly Ala Gly Tyr Ile Tyr Glu Asp Leu Thr Pro 245 250 255
Ala Glu Lys Lys Glu Arg Leu Thr Glu Ala Met Asp Arg Trp Ile Lys
Gly Met Met Glu Val Thr Ala Thr Arg Val Ser Ala Trp Asp Ala Val
Asn Glu Ala Ile Ser Gly Arg Asp Thr Asn Gly Asp Gly Phe Tyr Glu 290 295 300
Leu Glu Ser Ala Gln Trp Gly Ser Ser Asn Asn Phe Tyr Trp Gln Asp 305 310 315 320
Tyr Leu Gly Ser Gly Asp Tyr Val Arg Ile Val Ile Ala Lys Ala Arg
Lys Tyr Tyr Glu Glu Phe Gly Gly Thr Ala Pro Leu Arg Leu Phe Ile
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355 360 365
Ser Leu Ile His Trp Ile Gly Val Trp Glu Ser Asp Gly Val Thr Lys
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Ile Asp Gly Ile Gly Thr Gln Met His Val Ser Tyr Tyr Glu Asn Pro 395 400
Asp Ile Gln Ala Ser Lys Glu Lys His Tyr Val Gln Met Leu Gln Leu 405 410
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                                                          460
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465 470 480
Cys Met Thr Asp Ala Pro Gly Ala Ile Gly Thr Gly Trp Arg Gly Gly
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495
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130
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435
440
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35 40 45 Pro Ser Lys Glu His Glu Arg Ala Leu Ile Ala Gln His Phe Asn Ser 50 55 60 Leu Thr Ala Glu Asn Leu Met Lys Trp Glu Glu Ile Gln Pro Thr Glu 65 70 75 80 Gly Asn Phe Asp Phe Lys Ala Ala Asp Gln Leu Val Ala Phe Ala Glu 85 90 95 Gln His Gln Met Trp Met Ile Gly His Thr Ile Leu Trp His Glu Gln 100 105 110 Thr Pro Asp Trp Val Phe Gln Gly Leu Asp Gly Lys Pro Ala Ser Lys
115
120
125 Gln Leu Leu Leu Ala Arg Leu Thr Lys His Ile Gln Thr Val Val Gly
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140 Arg Tyr Gln Gly Arg Val Asn Gly Trp Asp Val Val Asn Glu Ala Leu 155 160 Asn Glu Asp Gly Ser Leu Arg Asp Thr Pro Trp Arg Arg Ile Leu Gly 165 170 175 Asp Asp Tyr Ile Ala Thr Thr Phe Ala Leu Val His Gln Val Asp Pro
180 185 190 Lys Ala Lys Leu Tyr Tyr Asn Asp Tyr Asn Leu Phe Lys Pro Glu Lys
195
200
205 Arg Ala Gly Val Leu Arg Ile Ile Gln Gln Leu Gln Gln Lys Asn Val 210 220 Pro Ile His Ala Ile Gly Glu Gln Ala His Tyr Gly Leu Asp Ser Pro 235 230 240 Ala Phe Lys Asp Val Glu Asp Ser Ile Asn Ala Phe Ala Ala Thr Gly
245 ______ 250 ____ 255 Leu Asp Val Met Leu Thr Glu Leu Glu Ile Ser Val Leu Pro Tyr Pro 260 265 270 Ser Gly Met Thr Gln Gly Ala Asp Ile Ser Gln His Gln Glu Leu Gln 275 280 285 Glu Gln Leu Asn Pro Tyr Arg Asp Gly Leu Pro Lys Ala Val Glu Gln 290 _ _ 300 _ _ Ala Trp Gln Gln Arg Tyr Leu Asp Leu Phe Ser Leu Leu Leu Arg Gln 305 310 320 Gln Gln Lys Leu His Arg Val Thr Phe Trp Gly Leu Asp Asp Gly Gln 325 335 Ser Trp Arg Asn Asn Phe Pro Met Arg Gly Arg Thr Asp Tyr Pro Leu 340 350 Leu Phe Asp Arg Lys Leu Gln Ala Lys Pro Leu Leu Ser Ala Leu Thr Ala Leu Ala Ala Asp Gln Thr Lys Ala Lys Pro Lys Met Asn Gln Leu 370 380 Gly Phe Ala Pro Thr Ser Thr Lys Leu Leu Ile Val Pro Gly Arg Gln 385 390 400 Ser Val Pro Phe His Val Leu Asp Thr Glu Thr Gly Gln Thr Val Leu 405 410 415 Gln Gly Gln Ser Ser Ala Ala Arg Phe Trp Pro Glu Ser Gly Glu Trp Val Ser Ala Ala Asp Phe Ser Ala Val Ile Thr Pro Gly Thr Tyr Gln
435
440
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485 490 495 Ala Ala Gly His Pro Asp Thr Lys Val Arg Val His Ala Ser Ala Ala 500 510 Ser Ala Ser Arg Pro Glu Gly Tyr Glu Leu Ser Ala Ala Lys Gly Trp 515 520 Tyr Asp Ala Gly Asp Tyr Asn Lys Tyr Val Val Asn Ser Gly Ile Thr 530 540 Page 213

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Ser Tyr Thr Leu Leu Gln Ala Trp Gln Asp Phe Pro Glu Phe Tyr Gln
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 Lys Thr Thr Ala Ala Ala Leu Asn Phe Ala Ala Val Leu Ala Lys Ala
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Gln Tyr Arg Gln Gln Ala Leu Leu Ala Trp Gln Trp Ala Gln Lys Asn
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Pro Gln Gln Ile Tyr Gln Gln Pro Lys Asp Val His Thr Gly Ala Tyr
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                                    680
                                                             685
Gly Asp Lys Gln Leu Ala Asp Glu Trp Ala Trp Ala Gly Ala Glu Leu
690 700
Tyr Leu Leu Thr Gly Glu Gln Ser Tyr Leu Gln Pro Leu Leu Ala Leu 705 710 720
Asp Thr Pro Ile Ser Ala Ala Ser Trp Ala Ser Val Ser Ala Leu Gly
725 730 735
Tyr Phe Ser Leu Ala Ser Ala Lys Gln Leu Glu Pro Ala Leu Arg Gln
740 745 750
Gln Val Gln Gln Lys Ile Gln Gln Ala Ala Gln Ile Leu Gln Glu
755 760 765
His Gln Thr Ser Ala Tyr Gln Val Ala Met Thr Lys Asn Asp Phe Val
Trp Gly Ser Asn Ala Val Ala Met Asn Lys Ala Met Leu Leu Tyr Gln 785 790 795 800
Ala Trp Lys Ile Ala Pro Lys Pro Glu Leu Leu Gln Ala Met Gln Gly
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Thr Gly Phe Gly Glu Gln Ser Pro Gln Gln Ile His His Arg Pro Ser
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Ala Val Gly Ala Leu Pro Ala Ala Ser Thr Leu Pro Ala Thr Thr Tyr
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Leu Asp Asp Trp Cys Ser Tyr Ala Thr Asn Glu Val Ala Ile Asn Trp
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                                                                                        540
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                                                                                              1080
                                                                                              1140
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35 40 45 Lys Lys Pro Ile Ala Gly Ile Glu Val Glu Ile Lys Gln Ile Arg His 50 55 60 Glu Phe Ala Phe Gly Ser Ala Met Asn Asp Gln Val Leu Phe Asn Gln 65 70 75 80 Gln Tyr Ala Asp Phe Phe Val Lys Tyr Phe Asn Trp Ala Val Phe Glu 90 Asn Glu Ala Lys Trp Tyr Ala Asn Glu Pro Gln Arg Gly Arg Ile Thr Tyr Glu Lys Ala Asp Ala Met Leu Asn Phe Ala Asp Arg His Gln Leu 115 120 125 Pro Val Arg Gly His Ala Leu Phe Trp Glu Val Glu Asp Ala Asn Pro 130 135 140 Ser Trp Leu Arg Ser Leu Pro Asn His Glu Val Tyr Glu Ala Met Lys 145 150 155 160 Asn Arg Leu Glu His Ala Gly Asn His Phe Lys Gly Arg Phe Arg His 165 170 175 Trp Asp Val Asn Asn Glu Met Met His Gly Ser Phe Phe Lys Asp Arg 180 185 190 Phe Gly Lys Asn Ile Trp Lys Trp Met Tyr Glu Glu Thr Lys Lys Ile 195 200 205 _ Asp Pro Gln Ala Leu Leu Phe Val Asn Asp Tyr Asn Val Ile Ser Tyr 210 215 220 Gly Glu His His Ala Tyr Lys Ala His Ile Asn Glu Leu Arg Gln Leu 225 230 235 240 Gly Ala Pro Ile Glu Ala Ile Gly Val Gln Gly His Phe Glu Glu Arg Val Asp Pro Val Ile Val Lys Glu Arg Leu Asp Val Leu Ala Glu Leu 260 270 Gly Leu Pro Ile Trp Val Thr Glu Tyr Asp Ser Val His Pro Asp Pro 275 280 285 Asn Arg Arg Ala Asp Asn Leu Glu Ala Leu Tyr Arg Val Ala Phe Ser 290 295 300 His Pro Ala Val Lys Gly Val Leu Met Trp Gly Phe Trp Ala Gly Ala 305 310 315 320 His Trp Arg Gly Glu Asn Ala Ala Ile Val Asn Tyr Asp Trp Ser Leu 325 330 335 Asn Glu Ala Gly Arg Arg Tyr Glu Lys Leu Leu Asn Glu Trp Thr Thr 340 345 350 Gln Arg Ile Glu Lys Thr Asp Ala Asn Gly His Val Arg Cys Pro Ala Phe His Gly Thr Tyr Glu Val Arg Ile Gly Lys Glu Ser Lys Met Leu

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Leu Asp Val Ile Leu Pro Gln Glu Gly
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<211> 1002
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Lys Gln Leu Leu Ile Asp His Val Asn Ser Ile Thr Ala Glu Asn His 35 40 45
Met Lys Phe Glu His Leu Gln Pro Glu Glu Gly Lys Phe Thr Phe Gln 50 60
Glu Ala Asp Arg Ile Val Asp Phe Ala Cys Ser His Arg Met Ala Val 75 80
Arg Gly His Thr Leu Val Trp His Asn Gln Thr Pro Asp Trp Val Phe 85 90 95
Gln Asp Gly Gln Gly His Phe Val Ser Arg Asp Val Leu Leu Glu Arg
Met Lys Cys His Ile Ser Thr Val Val Arg Arg Tyr Lys Gly Lys Ile
115 120 125
Tyr Cys Trp Asp Val Ile Asn Glu Ala Val Ala Asp Glu Gly Asp Glu
130 135 140
Leu Leu Arg Pro Ser Lys Trp Arg Gln Ile Ile Gly Asp Asp Phe Met
145 150 155 160
Glu Gln Ala Phe Leu Tyr Ala Tyr Glu Ala Asp Pro Asp Ala Leu Leu
165 170 175
Phe Tyr Asn Asp Tyr Asn Glu Cys Phe Pro Glu Lys Arg Glu Lys Ile
180 185 190
Phe Ala Leu Val Lys Ser Leu Arg Asp Lys Gly Ile Pro Ile His Gly
200 205
Ile Gly Met Gln Ala His Trp Ser Leu Thr Arg Pro Ser Leu Asp Glu
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Ile Arg Ala Ala Ile Glu Arg Tyr Ala Ser Leu Gly Val Val Leu His
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Asp Leu Ala Ala Pro Thr Ser Glu Met Ile Glu Arg Gln Ala Glu Arg
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Tyr Gly Gln Ile Phe Ala Leu Phe Lys Glu Tyr Arg Asp Val Ile Gln
275 280 285
Ser val Thr Phe Trp Gly Ile Ala Asp Asp His Thr Trp Leu Asp Asn
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Glu Ala Ala Pro His Ser Asn Ala Lys Phe Asp Arg Leu Arg Trp Ala
Ala Pro Glu Gly Phe Phe Ile Gly Ser Ala Ala Gly Gly Gly His 50 55 60
His Leu Glu Gln Asp Tyr Pro Asp Pro Phe Thr Phe Asp Lys Lys Tyr 65 70 75 80
Arg Lys Ile Leu Gly Gin Gln Phe Asn Ser Val Ser Ala Glu Asn Gln 90
```

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Met Lys Trp Glu Phe Ile His Pro Glu Arg Asp Gln Tyr Arg Phe Glu
100 105 110
Glu Ala Asp Ala Ile Val Glu Phe Ala Gln Arg Asn Arg Gln Ala Val
Arg Gly His Thr Leu Leu Trp His Ser Gln Asn Pro Glu Trp Leu Glu
130 135 140
Glu Gly Asp Phe Thr Lys Glu Glu Leu Arg Ala Ile Leu Lys Asp His
150 155 160
lle Asp Thr Val Val Gly Arg Tyr Ala Gly Lys Ile Gln Gln Trp Asp
165 170 175
Val Ala Asn Glu Ile Phe Asn Asp Gln Ala Glu Leu Arg Thr Asp Glu
180 185 190
Asn Ile Trp Ile Arg Glu Leu Gly Pro Glu Ile Val Ala Asp Ala Phe
195 200 205
Arg Trp Ala His Glu Ala Asp Pro Glu Ala Lys Leu Phe Leu Asn Asp 210 220
Tyr Asn Val Glu Gly Ile Asn Ala Lys Ser Asp Ala Tyr Tyr Glu Leu
225 230 235 240
Ala Gln Glu Met Leu Glu Gln Gly Val Pro Leu His Gly Phe Gly Ala
245 250 255
Gln Gly His Leu Ser Thr Arg Tyr Gly Phe Pro Gly Asp Leu Gln Gln 260 265 270
Asn Leu Gln Arg Phe Ala Asp Leu Gly Leu Glu Thr Ala Ile Thr Glu
275 280 285
Ile Asp Val Arg Met Asp Leu Pro Ala Ser Gly Lys Pro Thr Lys Glu 290 295 300
Gln Leu Arg Gln Gln Ala Asp Tyr Tyr Gln Gln Ala Leu Ser Ala Cys 305 310 315 320
Leu Ala Val Asn Asp Cys Asn Ser Phe Thr Ile Trp Gly Phe Thr Asp 325 330 335
Lys Tyr Ser Trp Val Pro Val Phe Phe Glu Gly Glu Gly Ser Ala Thr
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                                                                                                              660
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                                                                                                            1200
                                                                                                            1260
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1380

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Leu Val Cys Leu Ala Phe Gly Ser Ser Leu Thr Trp Gly Gln Cys Thr 35 40 45
Thr Phe Thr Thr Ser Thr Ile Arg Asn Cys Asp Gly Ile Asp Tyr Glu 50 60
Leu Trp Ser Gln Asn Asn Ser Gly Thr Thr Asn Met Gln Ile Thr Gly 65 70 _ _ _ 75 80
Gly Asn Ser Asn Pro Asn Gly Gly Thr Phe Glu Ala Thr Trp Ser Gly
85 90 95
Thr Ile Asn Val Leu Phe Arg Ala Gly Lys Lys Trp Gly Thr Ser Ser 100 105 110
Thr Ser Thr Pro Lys Thr Ile Gly Asn Ile Ser Leu Glu Phe Ala Ala 115 120 125
Thr Trp Ser Ser Val Asp Asn Val Lys Met Leu Gly Ile Tyr Gly Trp
130 140
Ala Tyr Tyr Pro Ser Gly Ser Glu Pro Thr Lys Thr Glu Ser Gly Gln
145 150 155 160
Ser Thr Asn Phe Ser Asn Gln Ile Glu Tyr Tyr Ile Ile Gln Asp Arg
165 _ 170 175
Gly Ser Tyr Asn Pro Ala Ser Gly Gly Thr Asn Ala Lys Lys Tyr Gly
180
185
190
Glu Gly Thr Ile Asp Gly Ile Ala Tyr Asp Phe Tyr Val Ala Asp Arg
Ile Gly Gln Ala Met Leu Thr Gly Thr Gly Asn Phe Lys Gln Tyr Phe 210 220
Ser Val Pro Lys Ser Thr Ser Ser His Arg Gln Ser Gly Thr Val Ser 225 230 235 240
Val Ser Lys His Phe Glu Ala Trp Glu Lys Ala Gly Met Lys Met Met 245 250 255
Asp Cys Arg Leu Tyr Glu Val Ala Met Lys Val Glu Ser Tyr Thr Gly 260 265 270
Ser Ala Asn Gly Asn Gly Ser Ala Lys Val Thr Lys Asn Leu Leu Thr
275 280 285
                                                        285
Ile Gly Gly Ser Ser Ser Asn Glu Phe Ser Leu Val Thr Asn Val Ser
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Pro Ala Ser Ala Gly Thr Val Ser Lys Ser Pro Asp Asn Ala Ser Tyr 305 310 315 320
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Lys Phe Val Gly Trp Glu Gly Asp Ala Ser Gly Ser Thr Ser Pro Thr 340 345 350
Ser Val Thr Met Ser Lys Asp Leu Thr Val Thr Ala Lys Phe Glu Leu 355 360 365
Val Ser Glu Glu Gly Ser Thr Asn Leu Ile Gln Asp Gly Asn Phe Pro 370 380
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Gln Gly Glu Asn Trp Gly Asn Ser Ala Ala Thr Thr Ser Val Ser Asn

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Lys Leu Thr Phe Lys Ala Arg Ala Asp Ala Ala Arg Lys Ile Glu Val
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                                                                                             300
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                                                                                             840
                                                                                             900
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                                                                                             960
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Gly Thr Val Asn Phe Cys Leu Gln Ser Gly Gly Arg Tyr Thr Ser Asn 50 55 60
 Trp Ser Gly Ile Asn Asn Trp Val Gly Gly Lys Gly Trp Gln Thr Gly 65 70 75 80
Ser Arg Arg Val Val Asn Tyr Ser Gly Ser Phe Asn Ser Pro Gly Asn 85 90 95
Gly Tyr Leu Thr Leu Tyr Gly Trp Thr Thr Asn Pro Leu Ile Glu Tyr
Tyr Ile Val Asp Asn Trp Gly Thr Tyr Arg Pro Pro Gly Gly Gln Gly
115
120
125
Phe Met Gly Thr Val Thr Ser Asp Gly Ala Thr Tyr Asp Val Tyr Arg
 Thr Gln Arg Val Asn Gln Pro Cys Ile Thr Gly Ser Ser Cys Thr Phe
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Tyr Gln Tyr Trp Ser Val Arg Gln Ser Lys Arg Thr Gly Gly Thr Ile
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Thr Thr Gly Asn His Phe Asp Ala Trp Ala Ser Tyr Gly Met Asn Leu
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Gly Ala His Asn Tyr Gln Ile Met Ala Thr Glu Gly Tyr Gln Ser Ser
195 200 205
Gly Ser Ser Asp Ile Thr Val Ser Glu Gly Ser Ser Ser Ser Ser Ser Ser 210 220
Gly Gly Gly Ser Ser Thr Ser Ser Ser Gly Gly Gly Gly Thr Lys Ser 225 235 240
Phe Thr Val Arg Ala Arg Gly Thr Ala Gly Gly Glu Ser Ile Thr Leu
245 250 255
Arg Val Asn Asn Gln Asn Val Gln Thr Trp Thr Leu Gly Thr Ser Met 260 265 270
Thr Asn Tyr Thr Ala Ser Thr Ser Leu Ser Gly Gly Ile Thr Val Ala
275 280 285
Tyr Thr Asn Asp Ser Gly Asn Arg Asp Val Gln Val Asp Tyr Ile Ile 290 295 300
Val Asn Gly Ser Thr Arg Gln Ser Glu Ala Gln Ser Tyr Asn Thr Gly 315 315 320
Leu Tyr Ala Asn Gly Ser Cys Gly Gly Gly Ser Asn Ser Glu Trp Met
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                                                                                                        180
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Gly Ser Gly Gly Asn Tyr Ser Val Asn Trp Ser Asn Thr Gly Asn Phe
50 60
Val Val Gly Lys Gly Trp Thr Thr Gly Ser Pro Phe Arg Thr Ile Asn 70 75 80
Tyr Asn Ala Gly Val Trp Ala Pro Asn Gly Asn Ala Tyr Leu Thr Leu
85 90 95
Tyr Gly Trp Thr Arg Ser Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser
Trp Gly Thr Tyr Arg Pro Thr Gly Thr Tyr Lys Gly Thr Val Tyr Ser
Asp Gly Gly Thr Tyr Asp Val Tyr Thr Thr Arg Tyr Asp Ala Pro
130
135
140
Ser Ile Asp Gly Asp Lys Thr Thr Phe Thr Gln Tyr Trp Ser Val Arg
Gln Ser Lys Arg Pro Thr Gly Ser Asn Ala Thr Ile Thr Phe Ser Asn 165 170
His Val Asn Ala Trp Lys Arg Tyr Gly Met Asn Leu Gly Ser Asn Trp
Ser Tyr Gln Val Leu Ala Thr Glu Gly Tyr Arg Ser Ser Gly Ser Ser 195 200 205
Asn Val Thr Val Trp
      210
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<211> 1404
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                                                                                                        420
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                                                                                                      1320
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1404

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100 105 110 Val Ser Ala Ala Asn Glu Pro Tyr Ser Ile Phe Asn Gln Thr Asp Gly
115 120 125 Thr Asp Arg Gln Asp Val Ile Leu Glu His Phe Asn Glu Met Thr Ala 130 135 140 Gly Asn Ile Met Lys Met Ser Tyr Val Tyr Ala Gly Gln Arg Ala Asn 145 _ 150 _ 155 _ 160 Gln Gln Pro Asp Gln Phe Asp Phe Ser Arg Ala Asp Glu Leu Val Gly
165 170 175 Phe Ala His Ala Asn Ser Val Lys Ile His Gly His Ala Leu Val Trp
180 185 190 His Ala Asp Tyr Gln Val Pro Gly Phe Met Gln Asn Tyr Asp Gly Asp 195 200 205 Phe Ala Glu Met Leu Ala Asn His Ala Arg Ser Val Val Glu His Phe 210 220 Asp Glu Glu Phe Pro Gly Thr Val Val Ser Trp Asp Val Val Asn Glu 225 230 235 240 Ala Ile Thr Asp Asn Phe Gly Thr Asp Thr Ash Gly Trp Arg Ser 245 250 255 Leu Phe Tyr Asn Ala Leu Pro Pro Ala Thr Glu Asp Asp Ile Pro Glu 260 265 270 Tyr Ile Arg Val Ala Phe Gln Ala Ala Arg Asp Ala Asn Pro Asp Ile 275 280 285 Asp Leu Tyr Tyr Asn Asp Tyr Asp Asn Thr Ala Asn Thr Asn Arg Leu 290 295 300 Asn Lys Thr Leu Gln Ile Ala Asp Ala Leu Ala Glu Asp Glu Leu Ile 305 310 315 320 Asp Gly Val Gly Phe Gln Met His Val Tyr Met Thr Tyr Pro Ser Leu 325 330 335 Ser His Phe Gln Asn Ala Phe Gln Glu Val Val Asp Arg Gly Leu Lys 340 350 Val Lys Ile Thr Glu Leu Asp Val Ser Val Val Asn Pro Tyr Gly Gln 355 360 365 Ser Thr Pro Pro Pro Gln Pro Val Tyr Asp Glu Ala Leu Ala Gly Ala 370 380 Gln Lys Lys Arg Phe Cys Asp Ile Thr Arg Val Tyr Leu Glu Thr Val 385 390 395 400 Pro Ala Glu Leu Arg Gly Gly Leu Thr Val Trp Gly Leu Ala Asp Asn 410 415 Glu Ser Trp Leu Met Gln Gln Phe Arg Asn Ala Thr Gly Ala Asn Tyr 420 425 430

Thr Asp Val Trp Pro Leu Leu Phe Asn Ala Asp Leu Ser Ala Lys Pro

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300
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                                                                                                                                                                                     480
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2280
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                                                                                                                                                                                 3300
                                                                                                                                                                                 3360
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                                                                                              Page 224
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Val Pro Val Thr Arg Arg Ile Thr Gly Ile Ser Pro Ser Phe Ala Gln
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Page 228

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1055

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Glu Thr Phe Glu Lys Asp Leu Leu Asn Asp Val Ile Pro Phe Ile Glu 1105 1115 1120 1120 Lys Asn Tyr Pro Val Lys Thr Gly Ala Glu Asn Arg Ala Leu Ala Gly
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Arg Pro Ala Gly Ser Gly Cys Val Thr Cys Asn Gly Gln Gln Phe Gly 200 205
Gln Val Phe Ser Ile Arg Gln Gly Met Arg Ser Glu Gln Pro Lys Thr
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Pro Thr Ile Val Asp Ile Phe Asp Leu Arg Gly Asn Lys Val Lys Ser
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Val Tyr Asn Val Ala Tyr Glu Gly Asp Tyr Ser Leu Lys Val Ser Gly 65 70 75 80
Arg Asn Ala Ser Trp Asp Gly Ala Val Ile Asp Leu Thr Asp Lys Leu
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Ser Ala Asn Val Ser Tyr Thr Val Ser Leu Phe Val Arg His Ser Asp
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Gln Lys Pro Gln Arg Phe Ser Val Tyr Ala Tyr Val Lys Asp Ser Ala
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Ser Glu Lys Tyr Ile Pro Val Val Asp Lys Val Ala Val Pro Asn Tyr
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Trp Lys Gln Leu Val Gly Lys Phe Thr Ile Asn Thr Ser Asn Pro Val
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Gln Lys Ile Gln Leu Leu Val Cys Val Pro Thr Asn Lys Ser Leu Glu
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Gly Val Val Lys Ser Thr Asn Phe Glu Ser Gly Thr Thr Glu Gly Trp
Gln Ala Arg Gly Thr Gly Ser Val Ala Gln Ile Ser Val Val Ser Thr
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Gln Lys Leu Thr Leu Thr Met Glu Arg Lys Asn Ala Asp Gly Ser Thr
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Ile Phe Tyr Ile Glu Ser Pro Asn Ala Thr Leu Ser Phe Tyr Ile Asp
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Asp Phe Thr Ala Val Asp Lys Asn Ala Pro Val Val Ala Pro Gly Ile 340 350
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Arg Gly Thr Gly Val Thr Val Ser Val Val Asn Thr Val Ala His Thr 370 380
Gly Ser Lys Ser Leu Tyr Val Thr Gly Arg Ser Gln Asn Trp His Gly 385 390 400
Ala Glu Ile Asp Leu Thr Asn Val Leu Glu Lys Gly Lys Glu Tyr Gln
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410
415
Phe Ser Val Trp Val Tyr Gln Asp Ser Gly Ser Asp Gln Lys Leu Thr
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730
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1380

1392

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120
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Ser Tyr Ala Lys Lys Pro His Ile Ser Ala Leu Asn Ala Pro Gln Leu 35 40 45
Asp Gln Arg Tyr Lys Asn Glu Phe Thr Ile Gly Ala Ala Val Glu Pro
Tyr Gln Leu Gln Asn Glu Lys Asp Val Gln Met Leu Lys Arg His Phe 70 75 80
Asn Ser Ile Val Ala Glu Asn Val Met Lys Pro Ile Ser Ile Gln Pro
85 90 95
Glu Glu Gly Lys Phe Asn Phe Glu Gln Ala Asp Arg Ile Val Lys Phe
100 105 110
Ala Lys Ala Asn Gly Met Asp Ile Arg Phe His Thr Leu Val Trp His 115 120 125
Ser Gln Val Pro Gln Trp Phe Phe Leu Asp Lys Glu Gly Lys Pro Met
Val Asn Glu Thr Asp Pro Val Lys Arg Glu Gln Asn Lys Gln Leu Leu
150
150
150
160
Leu Lys Arg Leu Glu Thr His Ile Lys Thr Ile Val Glu Arg Tyr Lys
165 170 175
Asp Asp Ile Lys Tyr Trp Asp Val Val Asn Glu Val Val Gly Asp Asp
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Gly Lys Leu Arg Asn Ser Pro Trp Tyr Gln Ile Ala Gly Ile Asp Tyr
195
200
205
     Lys Val Ala Phe Gln Thr Ala Arg Lys Tyr Gly Gly Asn Lys Ile
210 215 220
Lys Leu Tyr Ile Asn Asp Tyr Asn Thr Glu Val Glu Pro Lys Arg Ser 225 230 235 240
Ala Leu Tyr Asn Leu Val Lys Gln Leu Lys Glu Glu Gly Val Pro Ile
245 250 255
Asp Gly Ile Gly His Gln Ser His Ile Gln Ile Gly Trp Pro Ser Glu 260 265 270

Ala Glu Ile Glu Lys Thr Ile Asn Met Phe Ala Ala Leu Gly Leu Asp 285 280 285
Asn Gln Ile Thr Glu Leu Asp Val Ser Met Tyr Gly Trp Pro Pro Arg 290 295 300
Ala Tyr Pro Thr Tyr Asp Ala Ile Pro Lys Gln Lys Phe Leu Asp Gln 305 310 315 320
Ala Ala Arg Tyr Asp Arg Leu Phe Lys Leu Tyr Glu Lys Leu Ser Asp 325 330 335
Lys Ile Ser Asn Val Thr Phe Trp Gly Ile Ala Asp Asn His Thr Trp 340 345 350
Leu Asp Ser Arg Ala Asp Val Tyr Tyr Asp Ala Asn Gly Asn Val Val
Val Asp Pro Asn Ala Pro Tyr Ala Lys Val Glu Lys Gly Lys Gly Lys 370 380
Asp Ala Pro Phe Val Phe Gly Pro Asp Tyr Lys Val Lys Pro Ala Tyr
385 390 395 400
Trp Ala Ile Ile Asp His Lys
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<211> 1695
<212> DNA
<213> Unknown
<220>
<223> Obtained from an environmental sample.
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gcgtgggagg tccaggtgga gctcgctggg acgaccgtga acagcagcca cagcgcggcg
                                                                                             180
240
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                                                                                             360
                                                                                             420
                                                                                             480
                                                                                             540
                                                                                             600
660
                                                                                             720
                                                                                             780
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                                                                                             960
                                                                                            1020
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                                                                                            1140
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                                                                                            1260
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                                                                                            1440
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<220> <223> Obtained from an environmental sample.

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35 40 45 Ala Gly Thr Thr Val Asn Ser Ser His Ser Ala Ala Phe Ser Ser Thr
50 55 60 Gly Thr Arg Leu Val Ala Lys Pro Leu Ser Trp Asn Ala Thr Leu Ala 65 70 75 80 Pro Ala Ala Lys Thr Thr Phe Gly Phe Cys Ala Ala Ala Pro Ser Ala 85 90 95 Ala Ala Arg Pro Ser Val Val Gln Val Thr Ala Asn Gly Ser Ala Thr Gly Thr Gly Gly Thr Ser Gly Gly Gly Thr Gly Gly Ser Thr Ala Thr Gly Gly Ser Thr Ala Thr Gly Gly Ser Gly Gly Ser Thr Ala Gly Val Cys Ala Ala Thr Tyr Glu Ala Glu Ser Met Leu His Ser Thr Gly Asn 150 155 160 Ala Ile Ser Gly Gly Trp Asn Ile Tyr Ser Asn Gly Asn Ile Thr Ala 165 170 175 Thr His Ser Phe Ala Ala Gly Ser Asn Arg Leu Thr Val His Ala Lys
180
185
190 Gly Asp Gln Ala Asn Gly Ala Pro Ile Met Arg Val Ser Val Gly Asn 195 200 205 Thr Val Val Gly Glu Val Pro Val Pro Val Thr Val Trp Thr Pro Tyr 210 215 220 Cys Phe Asp Tyr Ala Ala Ala Ser Ala Gly Ala Gln Thr Val Lys Ile 225 230 235 240 Glu Phe Thr Asn Asp Tyr Asn Gly Gly Thr Gly Ala Asp Arg Asn Leu 245 250 255 His Val Asp Lys Val Ala Val Gln Cys Gly Ala Ser Cys Asn Ser Gly 260 265 270 Ser Gly Gly Thr Gly Gly Ser Ser Gly Ser Gly Gly Thr Ser Ala 275 280 285 Thr Gly Gly Ser Ala Ser Gly Gly Ala Ala Gly Thr Thr Cys Thr Asn 290 295 300 Val Arg Pro Thr Gly Thr Asp Trp Asp Ala Ala Thr Cys Asp Met Trp 305 310 315 320 Ala Ser Gln Thr Ser Glu Cys Ser Ala Ala Trp Met Ile Asp Asn His 325 330 335 Tyr Cys Asp Gln Ser Cys Gly Arg Cys Ser Gly Gly Ser Gly Thr Gly 340 345 Gly Thr Asn Thr Gly Gly Thr Gly Gly Gly Val Thr Pro Ser Thr Cys 355 Thr Glu Pro Asn Ser Gln Gln Cys Ser Thr Tyr Lys Val Gly Thr His 370 380 Cys Gly Leu Thr Tyr Glu Ile Trp Thr Asp Gly Ser Ala Gly Cys Met 385 390 395 400 Thr Asn Thr Ser Tyr Gly Phe Leu Ala Asn Trp Ser Gln Gly Asn Ala 405 410 415 Asn Tyr Leu Ala Arg Lys Gly Val Arg Pro Gly Ser Ser Arg Pro Val 420 _ 425 _ 430 _ Val Thr Tyr Ser Ala Asn Tyr Gln Pro Asn Gly Asn Ser Tyr Leu Gly
435
440
445 Ile Tyr Gly Trp Thr Gln Asn Pro Leu Val Glu Tyr Tyr Ile Ile Asp
450
460 Ser Trp Gly Ser Trp Arg Pro Pro Gly Thr Gln Ala Met Gly Thr Val 465 470 475 480 Page 240

```
Gln Val Asp Gly Gly Thr Tyr Asp Ile Tyr Arg Ser Glu Arg Val Asn
485 490 495
                                                              490
 Lys Pro Ser Ile Glu Gly Asn Lys Thr Phe Trp Gln Tyr Trp Ser Val
 Arg Thr Gln Lys Arg Thr Ser Gly Thr Ile Thr Val Ala Pro His Phe
                                               520
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 Ser Leu Val Val Glu Gly Tyr Asn Ser Ser Gly Ser Ala Asp Val Thr
545 550 556
 Val Ser Phe Arg
 <210> 319
<211> 1095
 <212> DNA
 <213> Unknown
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ccgttgcgcg tcctggcagc caaagccggg atcgcgttcg gcacggccgt cgacatgaac gcgtacaaca acgacgcgac ctaccgtgag ctcgtcggcc aggagttctc gagcgtcacg gccgagaacg tcatgaagtg gcagctcctc gagccgcagc gaggggtcta caactggggt ccggccgatc agctcgtcgc cgtagccaac gagaacggcc agaaggtgcg cgggcaacg
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acaccggacg agctccggca gctcctgagg aaccacatct tcacggtgat gcgccacttc
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                                                                                                                       480
                                                                                                                        540
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                                                                                                                       660
                                                                                                                       720
                                                                                                                       780
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cgcgccggac ggtag
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<210> 320
<211> 364
<212> PRT
<213> Unknown
<223> Obtained from an environmental sample.
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<222> (1)...(27)
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Ser Glu Asn Thr Ser Thr Asp Gln Pro Leu Arg Val Leu Ala Ala Lys
                                              40
Ala Gly Ile Ala Phe Gly Thr Ala Val Asp Met Asn Ala Tyr Asn Asn 50 60
ASP Ala Thr Tyr Arg Glu Leu Val Gly Gln Glu Phe Ser Ser Val Thr
                                 70
Ala Glu Asn Val Met Lys Trp Gln Leu Leu Glu Pro Gln Arg Gly Val
85 _ 90_ 95
Tyr Asn Trp Gly Pro Ala Asp Gln Leu Val Arg Val Ala Asn Glu Asn
100 105 110
Gly Gln Lys Val Arg Gly His Thr Leu Ile Trp His Asn Gln Leu Pro
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120
 Thr Trp Leu Thr Ser Gly Val Ala Ser Gly Glu Ile Thr Pro Asp Glu
130
140
 Lys Gly Glu Ile His Gln Trp Asp Val Ala Asn Glu Val Ile Asp Asp
Ser Gly Asn Leu Arg Asn Thr Ile Trp Leu Gln Asn Leu Gly Pro Ser
180 185 190
Tyr Ile Ala Asp Ala Phe Arg Trp Ala Arg Lys Ala Asp Pro Asp Ala
195 200 205
Ala Leu Tyr Leu Asn Asp Tyr Asn Val Glu Gly Pro Asn Ala Lys Ala 210 220
Asp Ala Tyr Tyr Ala Leu Val Lys Gln Leu Leu Ala Asp Asp Val Pro
225 230 235 240
Val Asp Gly Phe Gly Ile Gln Gly His Leu Gly Val Gln Phe Gly Phe 245 250 255
Trp Pro Ala Ser Ala Val Ala Asp Asn Met Gly Arg Phe Glu Ala Leu 260 270
Gly Leu Gln Thr Ala Val Thr Glu Ala Asp Val Arg Met Ile Met Pro
275 280 285
Pro Asp Glu Asp Lys Leu Ala Ala Gln Ala Arg Gly Tyr Ser Thr Leu 290 295 300
Val Gln Gly Cys Leu Met Ala Lys Arg Cys Arg Ser Phe Thr Val 315
                               Tyr Ser Trp Val Pro Gly Thr Phe Pro Gly Gln
330 335
Gly Ala Ala Asn Leu Leu Ala Glu Asp Phe Gln Pro Lys
340 345
Tyr Ala Val Gln Asp Asp Leu Ala Arg Ala Gly Arg
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<211> 1608
<212> DNA
 <213> Unknown
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                                                                                                               120
                                                                                                                180
                                                                                                                240
gagtggtccg accgcctcgg cttccgctgg cacaactggg gcgacgcccg catcgagggc aacgcccttg ttcgtgatcg ctgggtgcag gaactctccc accccatcta tctccacgcg gcgcgcgacg gatcttgcc gctgccgcca atcaccgccc tcccatccgc gaccccgtcg ctccagaccg tgttccagga cacactcts atcaccgcc ctccagacgt gagcagtc
                                                                                                                300
                                                                                                                360
                                                                                                               420
                                                                                                               480
accgataacg acgcaaccaa gaccgctctc atcaagaagc aattcaacac catcacgccc gagaatgttc tcaagtgggg gccggttcat cctgagccca accggttcaa cttcgaatcc accgatcgtt acgtggactt tggtggaag aaccggatgt tcatcgtcgg ccacaccctc gtctggcacc accaccc cgcctgggtg ttcaagatt cccaaggcca gccgctcgac
                                                                                                               540
                                                                                                               600
                                                                                                               660
                                                                                                               720
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                                                                                                               780
                                                                                                               840
cctagccaat ggcttaaaat catcggcccc gactacattg ccaaagcgtt tgcccttgcccacgccgccg accctgccgc tgaactgtat tacaacgatt acagtctcga tcatcccgcc
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                                                                                                               960
aagīgtīgtīg gtgcgātcīgc gctggtīgaag cagctccaga cgaātggcāt atccattīgcc
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gggattggca cgcagaccca cgtcggactc aacggacctt ccccccagtc ggtggatgat
                                                                                                              1080
tcattgacgg cctttggcca gctcggcgtg aaggtcatgg ttaccgaact cgacgttgat gtgctgcccg ccgccagcca aaatcaaaac gcggatctca accagcccgc cttgtccaat cccgccctca atcccgcct caatccctat cccgatgggc tgccgcaagc cgtccaggac
                                                                                                              1140
                                                                                                              1200
                                                                                                              1260
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                                                                                                              1320
                                                                                                              1380
cgtggccgcg tcaactatcc gctgctgttc gaccgtgcca gccagcccaa gcccgccttc
gatgcggtca ttcgcgtcgc caaggacccg ccgacggttt cgcacaatct cacccgctc
cacgatgcgg cgcgggtcct ggtcaatccg cacaagggct ggtaccacca ctacccggac
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1560
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                                                                                                              1608
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<210> 322 <211> 536 <212> PRT <213> Unknown <220>

<223> Obtained from an environmental sample.

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405 410 415 Ala Val Gln Asp Lys Leu Ala Ala Arg Tyr Ala Glu Leu Phe Ala Val 420 425 430 Phe Val Lys His Ala Asp Lys Ile Ser Arg Val Thr Phe Trp Cys Val 435 440 445 Thr Asp Gly Asp Ser Trp Leu Asn Asn Trp Pro Val Arg Gly Arg Val 450 455 460 Asn Tyr Pro Leu Leu Phe Asp Arg Ala Ser Gln Pro Lys Pro Ala Phe 465 470 475 480

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ASP Ala Val Ile Arg Val Ala Lys Asp Pro Pro Thr Val Ser His Asn
                                                                          490
Leu Thr Pro Leu His Asp Ala Ala Arg Val Leu Val Asn Pro His Lys 500 505
Gly Trp Tyr His His Tyr Pro Asp Asn His Ile Asn Lys Tyr Glu Ile
515
520
525
Ala Arg Asp Ala Asp Leu Thr Glu
530 535
<210> 323
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<212> DNA
<213> Unknown
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                                                                                                                                                   60
                                                                                                                                                 120
                                                                                                                                                 180
                                                                                                                                                 240
ttacaacctg cccgacatta cacatatcag tcatgggagc gaaaagcttg gattggttta tggattttga ctcgcgttca tccactgtat ttgtctgatt ttttagagaa attacatgta
                                                                                                                                                 300
                                                                                                                                                 360
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                                                                                                                                                 420
                                                                                                                                                 480
aggaaattga tgattcgtta tattcatcaa atattagcgg cgatggatga ccagcatttc
                                                                                                                                                 540
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                                                                                                                                                 600
                                                                                                                                                 660
                                                                                                                                                 720
780
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                                                                                                                                               1860
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                                                                                                                                               1980
                                                                                                                                               2040
                                                                                                                                               2100
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<210> 324
<211> 784
<212> PRT
<213> Unknown -
<223> Obtained from an environmental sample.
<400> 324
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Met Met Leu Asn Ala Arg Cys Ile Gln Leu Met Lys Leu Leu Leu Arg

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Gln Leu Lys Glu Glu Gly Val Pro Ile Asp Gly Ile Gly His Gln Ser
625 630 635 640
His Ile Gln Ile Gly Trp Pro Ser Glu Ala Glu Ile Glu Lys Thr Ile
645 650 655
Asn Met Phe Ala Ala Leu Gly Leu Asp Asn Gln Ile Thr Glu Leu Asp 660 670
Val Ser Met Tyr Gly Trp Pro Pro Arg Ala Tyr Pro Thr Tyr Asp Ala
675 680 685
Ile Pro Lys Gln Lys Phe Leu Asp Gln Ala Ala Arg Tyr Asp Arg Leu 690 695 700
Phe Lys Leu Tyr Glu Lys Leu Ser Asp Lys Ile Ser Asn Val Thr Phe 705 710 715 720
Trp Gly Ile Ala Asp Asn His Thr Trp Leu Asp Ser Arg Ala Asp Val
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Ile Ser Asn Ala Thr Leu Gln Asn Gln Asp Ala Thr Met Leu Asp Leu 50 55 60
Ile Lys Arg Glu Phe Asn Ala Ile Thr Ala Glu Asn Cys Met Lys Trp 65 70 75 80
Glu Pro Ile Arg Pro Gln Leu Asp Gln Trp Asn Trp Glu Leu Ala Asp
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Arg Phe Val Asp Phe Gly Val Lys Asn Lys Met Tyr Val Val Gly His
100 105 110
Thr Leu Ile Trp His Ser Gln Ala Pro Ala His Ile Tyr Leu Asp Ala
115 120 125
Asp Gly Lys Pro Asn Ser Arg Asp Ala Gln Leu Lys Val Met Glu Glu 130 140
His Ile Arg Thr Leu Ala Gly Arg Tyr Lys Gly Lys Ile Asp Ala Trp
155 160
Asp Val Val Asn Glu Ala Val Glu Asp Asp Gly Ser Trp Arg Gln Thr
165 170 175
Gly Trp Tyr Lys Asn Met Gly Glu Glu Tyr Ile Ala His Ala Phe Arg
180 185 190
Leu Ala Ala Glu Val Asp Pro Asn Ala Lys Leu Leu Tyr Asn Asp Tyr
195 200 205
Asn Glu Ala Val Pro Ala Lys Arg Asp Ala Ile Ile Arg Val Val Lys
Gly Val Gln Lys Ala Gly Ala Pro Ile His Gly Val Gly Met Gln Gly 225 230 235 240
His Met Ser Leu Ser His Pro Asp Phe Ala Glu Phe Glu Lys Ser Ile
245 250 255
Ile Glu Tyr Ala Lys Leu Gly Val Lys Val His Val Thr Glu Leu Asp
260 265 270
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290 295 300
Ala Lys Val Glu Glu Leu Ala Ala Arg Tyr Glu Ala Leu Phe Lys 305 310 315 320
Ile Leu Leu Arg His Arg Asp Lys Ile Glu Arg Val Thr Thr Trp Gly
Thr Asn Asp Ser Glu Thr Trp Leu Asn Gly Phe Pro Ile Pro Gly Arg
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                                                                                  360
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35 40 45
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His Gly Leu Gln Trp Phe Gly Asp Tyr Val Asn Lys Asp Ser Leu Lys 70 _ _ _ 75 80
Trp Leu Arg Asp Asp Trp Gly Ile Thr Val Phe Arg Ala Ala Met Tyr 85 90 95
Thr Ala Glu Gly Gly Tyr Ile Glu Asn Pro Ser Val Lys Asn Lys Val
Lys Glu Ala Val Glu Ala Ala Lys Glu Leu Gly Ile Tyr Val Ile Ile
115 120 125
Asp Trp His Ile Leu Asn Asp Gly Asn Pro Asn Gln Asn Lys Glu Lys
130 140
Ala Lys Glu Phe Phe Lys Glu Met Ser Ser Leu Tyr Gly Ser Thr Pro
145 150 160
Asn Val Ile Tyr Glu Ile Ala Asn Glu Pro Asn Gly Asp Val Asn Trp
165 170 175
Lys Arg Asp Ile Lys Pro Tyr Ala Glu Glu Val Ile Ser Val Ile Arg
180 185 190
Lys Asn Asp Pro Asp Asn Ile Ile Ile Thr Gly Thr Gly Thr Trp Ser
Gln Asp Val Asn Asp Ala Ala Asp Asp Gln Leu Lys Asp Ala Asn Val
210 220
Met Tyr Ala Leu His Phe Tyr Ala Gly Thr His Gly Gln Tyr Leu Arg
225 230 235 240
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Glu Trp Gly Thr Ser Asp Ala Ser Gly Asn Gly Gly Val Phe Leu Asp 260 265 270
Gln Ser Arg Glu Trp Leu Asn Tyr Leu Asp Asn Lys Lys Ile Ser Trp
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Pro Gly Ala Ser Lys Thr Gly Gly Trp Pro Leu Ser Asp Leu Ser
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                       340
 Ser Leu Ser Val Gln Tyr Arg Thr Gly Asp Gly Ser Val Asn Ser Asn
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 Asn Leu Lys Asn Val Thr Val Arg Tyr Trp Tyr Asn Thr Lys Asn Lys
385 390 395 400
 Gly Gln Asn Phe Asp Cys Asp Tyr Ala Lys Ile Gly Cys Ser Asn Val
 Thr His Lys Phe Val Thr Leu Gln Lys Pro Val Lys Gly Ala Asp Ala
420
430
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450 455 460
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2100

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Gly Tyr Gln Trp Ile Gly Asn Thr Thr Ile Ser Thr Glu Ser Trp Thr 135 Thr Ile Arg Gly Thr Trp Leu Pro Arg Ala Asp Ala Asn Ala Ser Glu 145 150 155 160 Leu Tyr Val Tyr Pro Glu Val Thr Pro Val Ala Gly Phe Asp Tyr Leu 165 170 175 Leu Asp Asp Leu Leu Ile Glu Arg Ala Ala Pro Val Asp Gly Gly Ala
180
185
190 Pro Gly Thr Val Val Tyr Thr Ala Gly Phe Glu Thr Asp Leu Asp Gly
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200
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565 570 575 Glu Val Pro Ile Asp Ala Val Gly His Gln Phe His Val Ser Leu Ala
580 585 590 Met Pro Ile Ala Asn Leu Arg Gly Ala Leu Glu Arg Phe Gln Asp Thr Gly Leu Ile Gln Gly Val Thr Glu Leu Asp Val Thr Val Gly Asn Asn 610 620 Pro Thr Glu Ala Leu Leu Val Glu Gln Gly Tyr Tyr Tyr Arg Asp Ala 625 630 635 640 Phe Arg Leu Phe Arg Glu Phe Thr Glu Asp Leu Tyr Ser Val Thr Val 655 655 Trp Gly Leu Thr Asp Asp Arg Ser Trp Arg Ser Ala Gln Ala Pro Leu 660 665 670

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Leu Pro Leu His Gln Ile Asp Gly Ala Gly Glu Phe Gln Leu Arg Trp
725 730 735
Ala Ala Asp His Leu Thr Val Phe Val His Val Thr Asp Gly Asp Glu
740 745 750
Val Glu Ile Val Leu Gly Asp Glu Thr Tyr Thr Val Ser Ser Asp Gly
755 760 765
Glu Gly Asp Leu Asp Ala Val Thr Ala Ala Gly Glu Asn Gly Ser Trp
770 780
Thr Ala Val Val Arg Val Pro Leu Thr Ala Glu Gln Gly Asp Thr Ala
785 790 795 800
Gln Phe Asp Leu Arg Ile Ile Asp Gly Ala Thr Thr Ser Gly Trp Asn
805 810 815
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Val Glu Val Val Glu Ala Ala Asp Arg Pro Thr Ile Asp Gly Glu Ile
835 840 845
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360
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                                                                                                                                                                                                      360
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                                                                                                                                                                                                   1260
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                                           600
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      610
                                      615
Ile Cys Thr Ser Thr Ala Ser Gly Trp Gly Trp Glu Asn Asn Arg Ser
635 640
Cys Ile Thr Thr Ser Thr Cys Asn Ser Gln Gly Pro Gly Gly Gly Gly 655
Val Val Cys Asn
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                                                                                                               240
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                                                                                                               360
                                                                                                               420
                                                                                                               480
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                                                                                                               660
                                                                                                               720
                                                                                                               780
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                                                                                                              960
                                                                                                             1020
cattggccca tcgaacggcc cgatgctcct cttcctttcg atatctatct caaggccaag
                                                                                                             1080
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<210> 356
<211> 374
<212> PRT
<213> Unknown
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<221> SIGNAL
<222> (1)...(21)
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Val Glu Ile Glu Ala Asn Ile Pro Ser Leu Lys Glu Val Cys Ala Ser
                                            40
Tyr Phe Glu Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Ser Ser Pro 50 55 60
Pro His Asp Ala Leu Leu Arg Lys His Phe Asn Cys Leu Val Ala Glu 65 70 75 80
Asn Val Met Lys Pro Ala Ser Ile Gln Pro Ser Glu Gly Tyr Phe Asn
85 90 95
Trp Thr Glu Ala Asp Lys Ile Val Asn Tyr Ala Lys Ala His Gly Met
100 105 110
Lys Leu Arg Phe His Thr Leu Val Trp His Asn Gln Val Pro Asp Trp
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<223> Obtained from an environmental sample. <221> SIGNAL

<222> (1)...(25) <400> 358 Met Asn Asn Phe

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100 105 110 His Thr Leu Val Trp His Ser Gln Thr Pro Gly Trp Val Phe Glu Asp Glu Ala Gly Asn Pro Leu Gly Arg Asp Glu Leu Ile Glu Arg Met Arg 130 140 130 Asp His Ile His Thr Val Val Gly Arg Tyr Arg Gly Arg Ile His Ala 145 150 160 Ser Pro Trp Tyr Arg Ile Ile Gly Glu Asp Tyr Leu Leu Lys Ala Phe 180 185 190 Glu Phe Ala His Glu Ala Asp Pro Asp Ala Glu Leu Tyr Tyr Asn Asp 195 200 205 Tyr Ser Leu Glu Asn Pro Ala Lys Arg Ala Gly Ala Val Arg Leu Val 210 215 220 Tyr Leu Gln Glu Asn Gly Ala Pro Ile His Gly Ile Gly Thr Gln
230 235 240 Gly His Tyr Ser Leu Asp Trp Pro Ser Leu Asp Glu Ile Glu Arg Thr 245 250 255 Ile Thr Asp Phe Ala Ala Leu Asp Val Asp Val Met Val Thr Glu Leu 260 265 270 Glu Ile Asp Val Leu Pro Ser Ala Phe Glu Tyr Gln Gly Ala Asp Ile 275 _ _ _ 280 285 Ala Met Arg Ala Glu Leu Glu Glu Arg Leu Asn Pro Tyr Pro Asp Glu 290 295 300 Leu Pro Ala Glu Val Asp Glu Ala Leu Thr Gln Arg Tyr Arg Asp Ile 305 310 315 320 Phe Glu Val Phe Leu Arg His Ser Asp Val Leu Thr Arg Val Thr Phe 325 330 Trp Gly Val Thr Asp Gly Asp Ser Trp Lys Asn Asn Trp Pro Val Pro 340 345 350 Gly Arg Thr Asn Tyr Pro Leu Leu Phe Asp Arg Glu Trp Gln Pro Lys 360 Pro Ala Phe Tyr Ser Val Ile Glu Val Ala Asp Glu Met Leu Asn Glu 370 380

<210> 359 <211> 2724 <212> DNA

<213> Unknown

<220> <223> Obtained from an environmental sample.

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145 150 155 160
Asp Val Val Asn Glu Ala Ile Asp Pro Ser Gln Pro Asp Gly Met Arg
165 170 175
Arg Ser Lys Trp Tyr Gln Ile Thr Gly Lys Asp Tyr Ile Lys Thr Ala
180 185 190
Phe Arg Val Ala Asp Asp Glu Leu Arg Lys Asn Gly Trp Arg Lys Glu 195 200 205
Gly Arg Gln Leu Tyr Ile Asn Asp Tyr Asn Thr His Asp Pro Thr Lys 210 220
Arg Glu Tyr Ile Trp Arg Leu Ile Asp Glu Leu Gln Thr Glu Gly Ile
225 230 240
Pro Val Asp Gly Val Gly His Gln Thr His Ile Asn Ile Glu Trp Pro
245 250 255
Pro Val Asn Gln Île Val Asp Ser Île Arg Phe Phe Gly Glu Lys Gly 260 265 270
Leu Asp Asn Gln Val Thr Glu Leu Asp Val Ser Ile Tyr Thr Asp Arg 275 280 285
     Ser Ser Tyr Gly Ser Tyr Gln Ala Ile Pro Gln Glu Val Phe Ile
290 295 300
Lys Gln Gly Asn Arg Tyr Lys Glu Leu Phe Glu Gly Leu Lys Ser Val 305 310 320
Lys Asn Tyr Leu Ser Asn Val Thr Phe Trp Gly Met Ala Asp Asp His 325 330 335
Thr Trp Leu Asn His Trp Pro Ile Glu Arg Pro Asp Ala Pro Leu Pro 340 345 350

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Ala Leu Lys Leu Ser Arg
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<210> 357
<211> 1155
<212> DNA
<213> Unknown
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<223> Obtained from an environmental sample.
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                                                                                                    240
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gaattcggtg aagcccacga catgttcatg ataggccata cgcttgtatg gcacagccag
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                                                                                                   1080
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<210> 358
<211> 384
<212> PRT
<213> Unknown
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Trp Arg Glu Leu Ile Phe Glu Arg Trp Gln Thr Asp Glu Thr Gly Val
Thr Pro Glu His Gly Ala Ile Tyr Val Arg Gly Phe Lys Gly Asp Tyr 545 550 560
Glu Ile Thr Val Lys Ala Gly Gly Gln Glu Val Arg Val Pro Tyr Thr
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130
135
140 Arg Glu Asp Asp Trp His Gly Ala Thr Phe Ser Val Gly His Leu Thr
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165 170 175 Glu Pro Asn Thr Val Val Lys Ile Thr Gly Lys Arg Glu Gly Glu Ser Ala Thr Tyr Glu Glu Tyr Thr Asp Val Gly Thr Ala Leu Ala Thr Asp 195 200 205 Gly Ser Trp Thr Glu Ile Thr Gly Thr Tyr Ile Pro Asp Ser Ala Ser 210 225 220 Pro Phe Glu Tyr Phe Ile Val Glu Thr Glu Glu Gly Pro Thr Val
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435 440 445 445 Asn Gly Leu Asp Lys Thr Asp Gly Leu Ile Gln Leu Leu Glu Arg Leu 450 460 Arg Asp Asn Asp Val Pro Ile Asp Gly Val Gly Phe Gln Met His Val 465 470 475 480 Leu Leu Asp Trp Pro Asp Ile Ser Thr Ile Arg Arg Ser Trp Glu Arg
485 490 495 Ala Leu Ala Val Asp Pro Asp Asp Arg Met Leu Leu Lys Ile Thr Glu
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Trp Lys Asp Ser Gly Ser Ala Thr Met Thr Leu Ala Ala Gly Gly Arg 40 Tyr Thr Ser Gln Trp Thr Asn Asn Thr Asn Asn Trp Val Gly Gly Lys
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120
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250 255 Gly Glu His Ile Asn Leu Arg Ile Gly Gly Asn Thr Val Ala Ser Trp
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405 410 415 Pro Val Thr Trp Phe Asn Gln Gly Asn Asn Val Val Ala Asn Ala Asn 420 425 430 Tyr Met Ala Gln Gln Leu Ser Val Gly Glu Val His Asn His Ser Tyr
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Arg Leu Thr Asn Gly Gln Val Ile Leu Met His Asp Gly Ser Tyr Thr 530 540 Asn Thr Asn Ala Ala Ile Ala Gln Ile Ala Ser Ser Leu Arg Ala Lys 545 550 560 Gly Leu Cys Pro Gly Arg Ile Asp Pro Ala Thr Gly Arg Ala Val Ala 565 570 575 Pro Ala Gly Gly Asn Thr Gly Gly Gly Thr Val Ser Ser Thr Arg

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165 _ _ _ 170 _ _ 175 Leu Asn Tyr Gly Pro Gln Ala Thr Ala Glu Gln Leu Gly Leu Asp Arg Phe Tyr Glu Gly Ala Ala Ala Asp Ala Ala Trp Arg Gln Ala Ala Glu 195 200 205 Gln Arg Ile Glu Glu Ile Arg Lys Ala Gly Met Ile Ile Val Ala Val 210 215 220 Thr Pro Asp Gly Glu Pro Ile Glu Gly Ala Glu Ile Arg Ala Lys Leu 225 230 235 240 Lys Arg His Ala Phe Gly Trp Gly Thr Ala Val Ala Ala Ser Arg Leu 255 _____ 255 ____ 255 Leu Gly Thr Gly Thr Asp Ser Glu Arg Tyr Arg Asn Phe Ile Arg Glu 260 265 270 Asn Phe Asn Met Ala Val Leu Glu Asn Asp Leu Lys Trp Gly Pro Phe 275 285 Glu Glu Asn Arg Ala Arg Ala Met Asn Ala Leu Arg Trp Leu His Glu 290 295 300 Asn Gly Ile Thr Trp Ile Arg Gly His Asn Leu Val Trp Pro Gly Trp 305 310 315 320 Arg Trp Met Pro Ser Asp Val Arg Asn Leu Ala Asn Asn Pro Glu Ala 325 330 335 Leu Arg Gln Arg Ile Leu Asp Arg Ile Arg Asp Thr Ala Thr Ala Thr 340 345 350 Arg Gly Leu Val Val His Trp Asp Val Val Asn Glu Pro Val Ala Glu 355 360 365 Asp Val Leu Asn Ile Leu Gly Asp Glu Val Met Ala Asp Trp Phe 370 375 380 Arg Ala Ala Lys Glu Cys Asp Pro Glu Ala Arg Met Phe Ile Asn Glu 385 390 395 Tyr Asp Ile Leu Ala Ala Asn Gly Ala Asn Leu Arg Lys Gln Asn Ala 405 410 Tyr Tyr Arg Met Ile Glu Met Leu Leu Lys Leu Glu Ala Pro Val Glu 420 430 Gly Ile Gly Phe Gln Gly His Phe Asp Thr Ala Thr Pro Pro Glu Arg Met Leu Glu Ile Met Asn Arg Tyr Ala Arg Leu Gly Leu Pro Ile Ala 450 ____ 455 ___ 460 ____ Ile Thr Glu Tyr Asp Phe Ala Thr Val Asp Glu Glu Leu Gln Ala Gln
465 470 475 480 Phe Thr Arg Asp Leu Met Ile Leu Ala Phe Ser His Pro Ala Val Ser 485 490

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Gly Tyr Trp Asn Gly Val Ile Gly Ala Asn Gly Gly Lys Ile Ser Phe
465 470 475 480
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565 570 575
Tyr Phe Arg Leu Thr Ile Pro Leu Ala Ala Asn Thr Ser Asn Ala Ile
580 585 590
Leu Val Glu Gly Arg Val Arg Val Ile Thr His Ser Asn Gly Cys Thr
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Tyr Gly Gly Tyr Thr Leu Ser Arg Thr Val Thr Ile Val Gln Ala Ser
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                                   615
                                                                620
Ser Pro Val Thr Leu Thr Pro Thr Ala Thr Pro Ser Pro Thr Ala Thr
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                                                          635
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                       645
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Thr Leu Thr Asn Thr Gly Gly Ser Ala Leu Asn Gly Trp Thr Leu Ala
                                         680
Tyr Ala Phe Pro Gly Asn Gln Thr Ile Ser Asn Ala Trp Asn Gly Thr
690 695 700
Ala Val Gln Ser Gly Ser Ser Val Ser Val Thr Asn Ala Gly Trp Asn
705
                             710
                                                          715
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Leu Cys His
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                                                                                                     900
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960

1020

1080 1140

1200

1242

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165 170 175 Val Cys Arg His Tyr Arg Gly Arg Ile Asn Glu Phe Asp Val Asn Asn 180 185 190 Glu Met Leu His Gly Asn Phe Phe Arg Ser Arg Leu Gly Asn Gly Ile
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200
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360 420

480

780

840 900 960

410 405

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<213> Unknown
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20 25 30
Asp Asn Leu Lys Asp Ala Phe Asp Gly Leu Phe Leu Ile Gly Thr Ala 35 40 45
Met Asn Thr Pro Gln Ile Thr Gly Gln Asp Thr Arg Thr Leu Glu Leu 50 55 60
Ile Lys Lys His Met Asn Ser Ile Val Ala Glu Asn Val Met Lys Ser
65 70 75 80
Gly Leu Ile Gln Pro Ser Glu Gly Glu Phe Asp Phe Ser Leu Ala Asp
                                                 90
                      85
Gln Phe Val Gln Phe Gly Val Asp Asn Asn Met His Ile Val Gly His
100 105 110
Thr Leu Ile Trp His Ser Gln Ala Pro Gly Trp Phe Phe Val Asp Glu
115 120 125
Asn Gly Asn Asp Val Ser Pro Glu Val Leu Lys Gln Arg Met Lys Asp
130 135 140
His Ile Tyr Thr Val Val Gly Arg Tyr Lys Gly Lys Val His Gly Trp
145 150 155 160
Asp Val Val Asn Glu Cys Ile Val Asp Asp Gly Ser Trp Arg Asn Ser
165 170 175
Lys Phe Tyr Gln Île Leu Gly Glu Asp Phe Val Lys Tyr Ala Phe Gln
180 185 190
Phe Ala Ser Glu Ala Asp Pro Asn Ala Glu Leu Tyr Tyr Asn Asp Tyr
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Ser Met Ala Leu Pro Gly Arg Arg Gln Gly Val Val Asn Met Val Lys 210 220
Asn Leu Gln Ala Gln Gly Ile Lys Ile Asp Gly Ile Gly Met Gln Gly 225 235 240
His Leu Met Ile Asp His Pro Ser Leu Glu Asp Phe Glu Thr Ser Leu
245 250 255
                                                  250
Leu Ala Phe Ala Asp Leu Gly Val His Val Met Ile Thr Glu Leu Asp
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Val Ser Val Leu Pro Phe Pro Thr Arg Asn Leu Gly Ala Asp Val Ser
275 280 285
Leu Asn Ile Ala Tyr Asn Thr Glu Leu Asn Pro Tyr Pro Asp Gly Leu
290 295 . 300
Pro Asp Asp Val Ala Gln Lys Leu His Asp Arg Trp Leu Asp Ile Tyr 305 310 315 320
Arg Leu Phe Ile Lys His His Asp Lys Ile Thr Arg Val Thr Thr Trp 325 330 335
Gly Thr Ala Asp Gly Met Ser Trp Lys Asn Asn Trp Pro Ile Arg Gly 340 345 350
Arg Thr Asp Phe Pro Leu Leu Phe Asp Arg Asp Phe Gln Pro Lys Pro 355
Val Val Ala Asp Ile Ile Lys Glu Ala Leu Ala Ala Lys Arg Lys
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<213> Unknown
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                                                                                                120
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Ser Pro Gln Lys Gly Val Ile Lys Met Ile Lys Arg Ala Phe Met Ile 20 25 30
Thr Leu Ala Ala Phe Leu Leu Phe Ala Leu Asn Ser Leu Pro Ile
His Ala Gly Ala Glu Gly Gly Glu Glu Lys Phe Thr Pro Lys Val Ile 50 55
                                                  Page 254
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Val Glu His Gly Phe Glu Asn Asn Asp Phe His Gly Trp Val Pro Arg
GĨy Gly Val Gly Thr IÎe Ser Ile Thr Asn GĨu Ala Ala His Ser GĨy
85 90
Ser Ser Cys Leu Lys Ile Thr Gly Arg Thr Gln Ala Trp His Met Pro
Arg Val Glu Ile Thr Lys Tyr Leu Glu Lys Gly Ala Lys Tyr Lys Ile
115 120 125
Glu Leu Tyr Val Lys Leu Pro Ala Gly Thr Ser Pro Arg Lys Phe Gln
130 135 140
Leu Ala Val Leu Thr Arg Tyr Leu Glu Gly Asn Gln Thr Arg Asp Lys
145 150 155 160
Glu Asp Ser Ile Ser Asp Glu Val Glu Val Thr Ala Asp Thr Trp Thr
165 170 175
Lys Val Glu Gly Glu Tyr Val Phe Asp Pro Ala Ala Ile Gly Ala Tyr
180 185 190
Val Tyr Pro Tyr Leu Lys Gly Asp Pro Ala Gly Ala Tyr Ala Pro Tyr
195 200 205
Leu Ile Asp Asp Phe Lys Ile Thr Thr Ile Ala Pro Ala Pro Lys Lys 210 220 220
Thr Ala Ala Thr Ala Ala Ala Lys Glu Ala Glu Glu Pro Leu Ile Glu
225 230 235 240
Thr Asp Ile Pro Ser Leu Lys Asp Val Cys Ala Ser Tyr Phe Glu Ile
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Gly Ala Ala Ile Glu Pro Tyr Glu Leu Phe Ser Lys Pro His Asp Gln 260 265 270
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                                                                                             240
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cgcatcgccg gcatgggcct taagctccgg gtcagcgaat tggacgtggg caccgacgga
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                                                                                             660
720
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tggcgccggg ccaactatcc cctgctcttc gacggcaaat tcaaccccaa gcccgccttc
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                                                                                             840
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<213> Unknown
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20 25 30
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Lys Asn Tyr Ile Lys Ala Val Phe Glu Tyr Thr Glu Thr Asn Tyr Pro
Gly Val Ile Val Ser Trp Asp Val Val Asn Glu Ala Ile Asp Asp Gly
100 105 110
Thr Asn Lys Leu Arg Gln Ser Asn Trp Phe Lys Thr Val Gly Glu Asp
115
120
125
Phe Val Leu Arg Ala Phe Glu Tyr Ala Arg Lys Tyr Ala Pro Glu Gly 130 140
Thr Leu Leu Tyr Tyr Asn Asp Tyr Asn Thr Ala Met Pro Gly Lys Leu
145 150 155 160
Asn Gly Ile Ala Asn Leu Leu Lys Ala Leu Ile Ala Glu Gly Asn Ile
165 170
                     165
                                                170
Asp Gly Tyr Gly Phe Gln Met His His Ser Val Gly Phe Pro Ser Met
                                          185
Glu Met Ile Ser Ala Ser Val Glu Arg Ile Ala Gly Met Gly Leu Lys
195 200 205
Leu Arg Val Ser Glu Leu Asp Val Gly Thr Asp Gly Asn Thr Glu Ser
210 215 220
Ser Phe Thr Lys Gln Ala Glu Lys Tyr Ala Ala Ile Met Arg Leu Leu
225 230 235 240
Leu Asp Tyr Lys Asp Gln Met Glu Ala Val Gln Val Trp Gly Leu Thr
245 250 255
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Lys Phe Asn Pro Lys Pro Ala Phe Tyr Ala Val Ala Asp Pro Tyr Ala 275 280 285
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aggaagaatg ggtggaggaa agaaggtcgt cagctctata tcaacgacta caacacccat
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35 40 45
Ala Ser Tyr Phe Glu Ile Gly Ala Ala Val Glu Pro Tyr Gln Leu Ser 50 55 60
Ser Pro Pro His Asp Ala Leu Leu Arg Lys His Phe Asn Cys Leu Val
Ala Glu Asn Val Met Lys Pro Ala Ser Ile Gln Pro Ser Glu Gly Tyr
85 90 95
Phe Asn Trp Thr Glu Ala Asp Lys Ile Val Asn Tyr Ala Lys Ala His
100 105 110
Gly Met Lys Leu Arg Phe His Thr Leu Val Trp His Asn Gln Val Pro
115
120
125
Asp Trp Phe Phe Ala Gly Asn Asp Lys Thr Arg Leu Leu Gln Arg Leu
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Glu Asn His Ile Arg Thr Ile Ile Lys Arg Tyr Gly Asp Lys Val Asp
145 150 155 160
Tyr Trp Asp Val Val Asn Glu Val Ile Asp Asp Asn Gly Gly Met Arg
165 170 175
Asn Ser Lys Trp Tyr Gln Ile Thr Gly Lys Asp Tyr Ile Lys Thr Ala
180 185 190
Phe Arg Val Ala Asp Asp Glu Leu Arg Lys Asn Gly Trp Arg Lys Glu 195 200 205
Gly Arg Gln Leu Tyr Ile Asn Asp Tyr Asn Thr His Asn Pro Thr Lys
210 215 220
Arg Glu Gly Ile Trp Arg Leu Ile Gln Glu Leu Arg Ala Glu Gly Ile
225 230 235 240
Pro Val Asp Gly Val Gly His Gln Thr His Ile Asn Ile Glu Trp Pro 245 250 255
Pro Val Ser Gln Ile Val Glu Ser Ile Arg Phe Phe Gly Glu Lys Gly 260 265 270
Leu Asp Asn Gln Val Thr Glu Leu Asp Val Ser Ile Tyr Thr Asn Asp 275 280 285
Lys Asp Ser His Gly Ser Tyr Gln Ala Ile Pro Gln Glu Val Phe Ile
290 295 300
Lys Gln Gly Asn Arg Tyr Lys Glu Leu Phe Glu Gly Leu Lys Ser Val
305 310 315 320
Lys Asn Tyr Leu Ser Asn Val Thr Phe Trp Gly Met Ala Asp Asp His 325 330 335
Thr Trp Leu Asn Arg Trp Pro Ile Glu Arg Pro Asp Ala Pro Leu Pro 340 345 350
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Ala Leu Lys Leu Ser Arg
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                                                                                        240
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                                                                                        300
gtgggcgtgg cggtctccgc cgccaacgag aacgacagca tcttcaacag tccggatgcc
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420

960

1020 1080 1140

1200

1260

1320

1347

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Ser Ala Gly His Phe Ser Ile Glu Pro Asp Phe Gln Leu Tyr Ser Leu
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Ala Asn Phe Pro Val Gly Val Ala Val Ser Ala Ala Asn Glu Asn Asp
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Ser Ile Phe Asn Ser Pro Asp Ala Ala Glu Arg Gln Ala Val Ile Ile
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                                  120
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Leu Gln Pro Ser Gln Gly Asn Phe Thr Phe Asp Asp Ala Asp Glu Leu
145 150 160
Val Asn Phe Ala Gln Ala Asn Gly Met Thr Val His Gly His Ser Thr
165 170 175
Ile Trp His Ala Asp Tyr Gln Val Pro Asn Phe Met Arg Asn Phe Glu
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Gly Asp Gln Glu Glu Trp Ala Glu Ile Leu Thr Asp His Val Thr Thr
195 200 205
Ile Ile Glu His Phe Pro Asp Asp Val Val Ile Ser Trp Asp Val Val 210 220
Asn Glu Ala Val Asp Gln Gly Thr Ala Asn Gly Trp Arg His Ser Val
225 230 235 240
Phe Tyr Asn Ala Phe Asp Ala Pro Glu Glu Gly Asp Ile Pro Glu Tyr 245 250 255
Ile Lys Val Ala Phe Arg Ala Ala Arg Glu Ala Asp Ala Asn Val Asp
260 265 270
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Gly Val Gly Phe Gln Met His Ala Tyr Met Asp Tyr Pro Ser Leu Thr
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370 375 380
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385 390 395 400
 Trp Leu Met Gln Gln Phe Arg Asn Ala Thr Gly Ala Asp Tyr Asp Asp 405 415
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100 105 110 Ile Glu Gln Leu Arg Lys Arg Asn Val Glu Ile Glu Val Val Asp Leu
115 120 125 Asn Gly Asn Pro Leu Pro Gly Ala Thr Val Arg Ala Val Gln Arg Thr His Gln Phe Gly Phe Gly Thr Ala Ile Asn Arg Thr Ala Leu Ser Asn 145 150 155 160 Pro Val Tyr Ala Asp Phe Val Lys Asn Arg Phe Glu Trp Val Thr Phe
165 170 175 Glu Asn Glu Ala Lys Trp Leu Trp Asn Glu Ala Val Gln Gly Arg Val Tyr Tyr Arg Glu Ala Asp Gln Leu Leu Glu Phe Ala Arg Gln Asn Gly
195 200 205 Lys Val Arg Gly His Asn Leu Phe Trp Glu Ala Glu Lys Tyr Gln 210 215 220 Pro Gln Trp Val Lys Ser Leu Thr Gly Ala Ala Leu Lys Glu Ala Ile 225 230 235 240 Asp Asn Arg Leu Asn Ser Ala Val Leu His Phe Lys Gly Asn Phe Leu 245 250 255 His Trp Asp Val Asn Asn Glu Met Phe His Gly Ser Phe Phe Lys Asp 260 265 270 Arg Leu Gly Glu Glu Ile Trp Thr Tyr Met Tyr Lys Arg Thr Arg Glu 275 280 285 Leu Asp Pro Gly Val Lys Leu Phe Val Asn Asp Tyr Asn Phe Ile Glu 290 295 300 Tyr Pro Pro Glu Arg Asp Tyr Asn Gln Val Ile Gln Ala Leu Ile Asp 305 310 315 320 Arg Gly Met Pro Ile Asp Gly Ile Gly Ala Gln Gly His Phe Asn Gly 325 330 335 Val Ile Asp Pro Leu Phe Val Lys Gly Arg Leu Asp Lys Leu Ala Glu 340 ______345 ____350 Leu Asn Leu Pro Ile Trp Ile Thr Glu Phe Asp Ser Thr His Lys Asp 355 360 365 Glu Arg Val Arg Ala Asp Asn Leu Glu Lys Met Tyr Arg Leu Ala Phe 370 380 Ala His Pro Ala Val Glu Gly Ile Val Met Trp Gly Phe Trp Ala Gly 385 _____ 390 ____ 395 ____ 400 Ser His Trp Lys Gly Thr Asp Gly Ala Ile Val Asn Gln Asp Trp Thr
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410
415 Leu Asn Ala Ala Gly Gln Arg Tyr Gln Gln Leu Met Asp Glu Trp Thr Thr Val Val Glu Gly Thr Thr Asp Gln Arg Gly Met Phe Ser Phe Arg
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445 Gly Phe His Gly Thr Tyr Asp Met Leu Val Asp Tyr Pro Gly Ala Ala 450 455 460 _ ____ Ala Val Lys Gln Ser Phe Thr Leu Glu Pro Gly Ser Gly Asn Ala Lys 465 470 475 480 Leu His Ile Pro Phe Asp Val Gln Asp Lys Ser Ile Pro Glu Ala Pro 485 490 495 Ala Lys Leu Ser Ala Ala Ala Ala Asp Ser Gln Val Met Leu Ser Trp 500 505

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Phe Thr His Ile Gly Leu Val Asn Arg Lys Asp Tyr Tyr Tyr Val Val 545 550 560
Ser Ala Ser Asn His Leu Gly Glu Ser Pro Asp Ser Ala Pro Ile Arg
565 570 575
Ala Thr Pro Arg Ala Ala Gly Glu Leu Gln Thr Asn Leu Val Leu Gln
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Tyr Arg Ser Ala Asp Gly Asp Asn Asn Tyr Gln Met Lys Pro Gln Phe 595 600 605
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Ile Arg Tyr Tyr Phe Thr Pro Glu Ser Thr Gln Pro Val Asp Thr Arg 625 630 635
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Pro Pro Ser Asp Ala Ala Ala His Ala Tyr Val Glu Leu Ser Phe Leu 660 670
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675 680 685
Leu Arg Île Phe Asn Ser Asp Gly Ser Ser Phe Asp Lys Thr Asn Asp 690 695 700
Tyr Ser Phe Asp Pro Thr Lys Lys Ala Tyr Thr Ala Trp Glu Lys Val
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480
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<213> Unknown
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1 5 10 15
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Arg Glu Asn Ser Leu Phe Tyr Gln Lys Leu Gly Lys Asp Phe Val Ala
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115 120 125
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Ser Cys Leu Leu Glu Leu Val Asp Glu Leu Leu Glu Ala Asp Val Pro
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                                                 155
The Thr Gly Val Gly Phe Gln Met His Val Gln Ala Thr Trp Pro Ser
165 170 175
Asn Ala Asn Ile Gly Lys Ala Phe Lys Ala Ile Ala Asp Arg Gly Leu
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Lys Val Lys Ile Ser Glu Leu Asp Val Pro Val Asn Asn Pro Tyr Gly
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    Thr Asn Phe Pro Gln Tyr Ser Ser Phe Thr Ala Glu Ala Ala Glu
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Asn Trp Glu Thr Pro Thr Val Val Ala Pro Val Ile Gln Thr Arg Thr
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80
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165 170 175 Arg Lys His Ile Ser Thr Val Val Gly Arg Tyr Lys Gly Arg Ile Lys
180

Glu Trp Asp Val Val Asn Glu Ala Ile Asn Asp Gly Pro Gly Val Leu
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200

205

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Pro Gln Pro Ile Val Leu Gly Pro Gly Asp Lys Pro Ala Phe Pro Pro 820 825 830
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Ile Lys Leu Val Glu Tyr Pro Ser Ala Thr Val Gly Thr Thr Arg Lys
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855
860
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1080

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aactcttacg tcgcgcttta cggctggacc agaaacccat tggttgagta ctacgtgatt
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Asn Ser Gln Gly Thr His Asp Gly Phe Phe Tyr Ser Phe Trp Lys Asp 45
Ser Gly Asn Ala Ser Met Asn Leu Leu Ala Gly Gly Arg Tyr Gln Ser 50 55 60 Ser Trp Asn Thr Gly Thr Asn Asn Trp Val Gly Gly Lys Gly Trp Asn 65 70 75 80
Pro Gly Thr Asn Asn Arg Val Ile Asn Tyr Ser Gly Tyr Tyr Gly Val
85 90 95
Asp Asn Ser Gln Asn Ser Tyr Val Ala Leu Tyr Gly Trp Thr Arg Asn 100 105 110
Pro Leu Val Glu Tyr Tyr Val Ile Glu Ser Tyr Gly Ser Tyr Asn Pro
115 120 125
Ala Ser Cys Ser Gly Gly Thr Asp Phe Gly Ser Phe Gln Ser Asp Gly
130 140
Ala Thr Tyr Asn Val Arg Arg Cys Gln Arg Val Gln Gln Pro Ser Ile
145 150 160
Asp Gly Thr Gln Thr Phe Tyr Gln Tyr Phe Ser Val Arg Asn Pro Lys
165
170
175
Lys Gly Phe Gly Asn Ile Ser Gly Thr Ile Thr Phe Ala Asn His Val
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Asn Tyr Trp Arg Ser Arg Gly Met Asn Leu Gly Asn His Asp Tyr Gln
195 200 205
Val Leu Ala Thr Glu Gly Tyr Arg Ser Thr Gly Ser Ser Asp Leu Thr
210 215 220
The Ser Gln Gly Ala Ser Asn Asn Gly Gly Gly Ser Ser Ser Ser 225 235 240
Ala Pro Ser Ala Gly Gly Gly Ser Lys Thr Ile Val Val Arg Ala Arg 245 250 255
Gly Thr Thr Gly Gln Glu Gln Ile Arg Leu Arg Val Asn Asn Thr Ile 260 265
Val Gln Thr Trp Thr Leu Ser Thr Thr Met Arg Asp Tyr Thr Val Asn 275 280 285
Thr Asn Leu Ala Gly Gly Ser Leu Val Glu Tyr Phe Asn Asp Ser Gly 290 295 300
Asn Arg Asp Val Gln Val Asp Tyr Ile Ser Val Asn Gly Asn Val Arg
305 310 315 320
Gln Ser Glu Asn Gln Thr Tyr Asn Thr Gly Val Tyr Gln Asn Gly Ala 325
Cys Gly Gly Gly Asn Gly Arg Ser Glu Trp Leu His Cys Asn Gly Ala 345
The Gly Tyr Gly Asn Tlo
Ile Gly Tyr Gly Asp Ile
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                                                                                                                        360
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gatatcggcg tatccaagaa caatcaggaa aactatgaca aacaggccaa acgctacaag
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                                                                                                                      1020
                                                                                                                      1080
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Asp Gln Gly Gly His Pro Thr Leu Ser Phe Glu Ile Arg Asn Phe
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Arg Leu Thr Ala Pro Glu Gly Ile Ala Pro Pro Lys Ala Thr Glu Glu
50 60
 Pro Ala Asp Ala Ala Glu Ala Thr Pro Val Pro Ala Leu Ser Glu Ile
65 _ 70 _ 75 _ 80
 Pro Gly Leu Lys Asp Val Tyr Ala Asp Tyr Phe Asp Phe Gly Ala Ala
Ala Pro Gln Tyr Ala Phe Gly Leu Gly Gln Thr Gln Leu Gln Asp Leu 100 110
Met Ile Ser Gln Phe Ser Ile Leu Thr Pro Glu Asn Glu Leu Lys Pro
115 120 125
Asp Ser Val Leu Asp Val Gln Thr Ser Lys Lys Leu Ala Ala Glu Asp
Glu Thr Ala Val Ala Ile Arg Leu Asn Ala Ala Thr Pro Leu Leu Lys
145 150 160
Phe Ala Gln Lys Asn Gly Ile Lys Val His Gly His Val Leu Val Trp
165 170 175
His Ser Gln Thr Pro Glu Ala Phe Phe His Glu Gly Tyr Asp Thr Lys
180
185
190
Lys Pro Tyr Val Thr Arg Glu Val Met Leu Gly Arg Leu Glu Asn Tyr 200 205
Ile Arg Glu Val Leu Thr Gln Thr Glu Glu Gln Phe Pro Gly Val Ile 210 220
Val Ser Trp Asp Val Val Asn Glu Ala Ile Asp Asp Gly Thr His Trp 225 235 240
Leu Arg Lys Thr Ser Ser Trp Tyr Lys Val Val Gly Glu Asp Phe Leu 245 250 255
Asn Arg Ala Phe Glu Tyr Ala Arg Lys Tyr Ala Ala Glu Gly Val Leu
260 265 270
Leu Tyr Tyr Asn Asp Tyr Ser Thr Ala Asn Ser Ala Lys Leu Met Gly 275 280 285
The Thr Lys Leu Leu Lys Gln Leu Ile Pro Asp Gly Asn Ile Asp Gly 290 295 300
Tyr Gly Phe Gln Met His His Asp Leu Gly Trp Pro Ser Ile Asp Leu 305 310 320
Met Ala Ala Ala Val Lys Gln Ile Ala Gly Leu Gly Leu Lys Leu Arg
Val Ser Glu Leu Asp Ile Gly Val Ser Lys Asn Asn Gln Glu Asn Tyr
340 345 350
Asp Lys Gln Ala Lys Arg Tyr Lys Glu Met Leu Asn Leu Met Leu Gln 355 360 365
Tyr Ala Asp Gln Thr Glu Ala Val Gln Val Trp Gly Leu Thr Asp Asn 370 380
Met Ser Trp Arg Thr Gly Lys Tyr Pro Leu Leu Phe Asp Ser Ala Ala 385 390 400 Lys Pro Lys Lys Ala Phe Phe Ala Val Ile Glu Ala Ala Glu Glu 405 410
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<211> 1539
<212> DNA
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aagggggtgt ttacttttga acaggcggac atgatggtgg acgcggtatt ggagcgggga
                                                                                                 60
                                                                                                120
                                                                                                180
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                                                                                                240
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                                                                                                300
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                                                                                                360
                                                                                                420
                                                                                                480
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                                                                                                540
                                                                                                600
                                                                                                660
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                                                                                                720
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gattcaaacc agacagagcg gcagcgggtg gaacagggcc tggtctatgc cgctttgttt
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accattttcc gggaacacgc ggcaaacata ggccgggtaa ctttttgggg acttgacgac
                                                                                                 840
                                                                                                 900
ggggcaagct ggcgttccgc ggcgagtccc tgcctctttg ataaaaacct caacgcaaaa
                                                                                                 960
cctgcctttt acgcggtcct ggacccggat tcctttattg cggaaaacag cgccctgctg
atcagggaag cgaaagaggg agaggcttat tatggtacgc ctgctttagg cgccgtccct gatccctct gggacagggc gccttccctc ccggtggatc agtacctcat ggcctggcag
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                                                                                                1080
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gttgaaaacg cggaaataaa caaggacagt tccaacagct acgaacagga ttcggtcgaa
                                                                                                1140
                                                                                                1200
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                                                                                                1260
                                                                                                1320
                                                                                                1380
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                                                                                                1440
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<223> Obtained from an environmental sample.

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115 120 125 Ala Ser Leu Arg Gln Ser Pro Trp Tyr Lys Ala Ile Gly Pro Asp Tyr 130 135 140 Val Glu Leu Val Phe Lys Ala Ala Arg Glu Ala Asp Pro Glu Ala Lys 145 150 155 160 Leu Tyr Tyr Asn Asp Tyr Asn Leu Asp Asn Arg Asn Lys Ala Leu Ala 165 170 175 Val Tyr Asn Met Val Arg Glu Leu Asn Glu Lys Asn Pro Asn Pro Gly 180 185 190 Gly Arg Pro Leu Ile Asp Gly Val Gly Met Gln Gly His Tyr Arg Leu 200 Asn Thr Asn Thr Asp Asn Val Arg Leu Ser Leu Glu Arg Phe Ile Ser 215 220 Leu Gly Val Glu Val Ser Ile Thr Glu Leu Asp Ile Gln Ala Gly Ser 235 240 Asp Ser Asn Gln Thr Glu Arg Gln Arg Val Glu Gln Gly Leu Val Tyr
245 _ 250 _ 255 Ala Ala Leu Phe Thr Ile Phe Arg Glu His Ala Ala Asn Ile Gly Arg Val Thr Phe Trp Gly Leu Asp Asp Gly Ala Ser Trp Arg Ser Ala Ala 275 280 285 Ser Pro Cys Leu Phe Asp Lys Asn Leu Asn Ala Lys Pro Ala Phe Tyr 290 295 300 Ala Val Leu Asp Pro Asp Ser Phe Ile Ala Glu Asn Ser Ala Leu Leu 305 310 315 320 Ile Arg Glu Ala Lys Glu Gly Glu Ala Tyr Tyr Gly Thr Pro Ala Leu 325 330 335 Gly Ala Val Pro Asp Pro Leu Trp Asp Arg Ala Pro Ser Leu Pro Val Asp Gln Tyr Leu Met Ala Trp Gln Gly Ala Ser Gly Arg Ala Lys Val Page 289

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Leu Trp Asp Glu Lys Asn Leu Tyr Val Leu Val Arg Val Glu Asn Ala
                            375
Glu Ile Asn Lys Asp Ser Ser Asn Ser Tyr Glu Gln Asp Ser Val Glu
                       390
                                               395
Ile Phe Ile Asp Glu Asp Asn Arg Lys Ser Ser Phe Phe Arg Glu Asp
                  405
                                          410
Asp Gly Gln Tyr Arg Val Asn Phe Ala Asn Glu Ala Gly Phe Asn Pro
Ser Ser Ala Gly Ala Gly Phe Val Ser Ala Ala Ala Val Asp Gly Lys
Ser Tyr Thr Val Thr Met Lys Ile Pro Phe Lys Thr Ile Val Pro Gly
450 455 460
Ala Gly Thr Arg Ile Gly Phe Asp Val Gln Ile Asn Gly Ala Ser Ala
465 470 480
Arg Gly Ile Arg Glu Ser Val Ala Val Trp Asn Asp Thr
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                   485
Ser Phe Gln Asp Thr Ser Gly Tyr Gly Val Leu Arg Leu Val Lys Lys 500 505
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                                                                                  180
gccttttcgg tagtgggtaa tgcttatttg gctctttatg ggtggacgag aaattcactc atagaatatt acgtcgttga tagctggggg acttatagac ctactggaac ttataaaggc
                                                                                  240
                                                                                  300
actgtgacta gtgatggagg gacttatgac atatacacga ctacacgaac caacgcacct
tccattgacg gcaataatac aactttcacc cagttctgga gtgttaggca gtcgaagaga
                                                                                  360
                                                                                  420
ccgattggta ccaacaatac catcaccttt agcaaccatg ttaacgcctg gaagagtaaa
                                                                                  480
ggaatgaatt tggggagtag ttggtcttat caggtattag caacagaggg ctatcaaagt
                                                                                  540
agtgggtact ctaacgtaac ggtctggtaa
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<210> 376
<211> 189
<212> PRT
<213> Artificial Sequence
<220>
<223> Synthetically generated polypeptide
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Ile Gly Thr Val Asn Ala Thr Asn Gly Ser Asp Gly Asn Tyr Ser Val
Ser Trp Ser Asn Cys Gly Asn Phe Val Val Gly Lys Gly Trp Thr Thr
Gly Ser Ala Thr Arg Val Ile Asn Tyr Asn Ala His Ala Phe Ser Val
Val Gly Asn Ala Tyr Leu Ala Leu Tyr Gly Trp Thr Arg Asn Ser Leu 65 _ 70 _ 75 _ 80
Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly Thr Tyr Arg Pro Thr Gly
Thr Tyr Lys Gly Thr Val Thr Ser Asp Gly Gly Thr Tyr Asp Ile Tyr
Thr Thr Arg Thr Asn Ala Pro Ser Ile Asp Gly Asn Asn Thr Thr
Phe Thr Gln Phe Trp Ser Val Arg Gln Ser Lys Arg Pro Ile Gly Thr
Asn Asn Thr Ile Thr Phe Ser Asn His Val Asn Ala Trp Lys Ser Lys
145 150 155 160
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175 175
Gly Tyr Gln Ser Ser Gly Tyr Ser Asm Val Thr Val Trp
<210> 377
<211> 570
<212> DNA
<213> Artificial Sequence
<223> Synthetically generated polynucleotide
<400> 377
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                                                                                          60
                                                                                         120
                                                                                         180
gccttttcgg tagtgggtaa tgcttatttg gctctttatg ggtggacgag aaatccactc atagaatatt acgtcgttga tagctggggg acttatagac ctactggaac ttataaaggc actgtgacta gtgatggagg gacttatgac atatacacga ctacacgaac caacgcacct tccattgacg gcaataatac aactttcacc cagttctgga gtgttaggca gtcgaagaga
                                                                                         240
                                                                                         300
                                                                                         360
                                                                                         420
ccgattggta ccaacaatac catcaccttt agcaaccatg ttaacgcctg gaagagtaaa
                                                                                         480
ggāatgāātt tggggagtag ttggtcttat cāggtattag caacagaggg ctatcaaagt
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<210> 378
<211> 189
<212> PRT
<213> Artificial Sequence
<220>
<223> Synthetically generated polypeptide
<400> 378
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Gly Ser Ala Thr Arg Val Ile Asn Tyr Asn Ala His Ala Phe Ser Val
Val Gly Ash Ala Tyr Leu Ala Leu Tyr Gly Trp Thr Arg Ash Pro Leu
65 70 75 80
Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly Thr Tyr Arg Pro Thr Gly
                                             90
Thr Tyr Lys Gly Thr Val Thr Ser Asp Gly Gly Thr Tyr Asp Ile Tyr 100 105 110
Thr Thr Thr Arg Thr Asn Ala Pro Ser Ile Asp Gly Asn Asn Thr Thr
                                   120
                                                            125
Phe Thr Gln Phe Trp Ser Val Arg Gln Ser Lys Arg Pro Ile Gly Thr
                              135
Asn Asn Thr Ile Thr Phe Ser Asn His Val Asn Ala Trp Lys Ser Lys
                         150
                                                  155
                                                                           160
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165 170 175
Gly Tyr Gln Ser Ser Gly Tyr Ser Asn Val Thr Val Trp
<210> 379
<211> 570
<212> DNA
<213> Artificial Sequence
<223> Synthetically generated polynucleotide.
<400> 379
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                                                                                         60
                                              Page 291
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<210> 380 <211> 189 <212> PRT <213> Artificial Sequence

V

<220>
<223> Synthetically generated polypeptide.